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# STUDIES UPON SOME CHROMOGENIC FUNGI WHICH DISCOLOR WOOD.\*

BY GEORGE GRANT HEDGCOCK.

## GENERAL REMARKS.

The discoloration of unpainted woodwork exposed to moisture and to oxidation by the air is a familiar phenomenon. Of course much of this discoloration is due to the dirt and soot which accumulate on the surface of boards in addition to the chemical changes which take place through weathering. There is also a class of stains of an entirely different nature and more striking to the eye. These occur on freshly sawn lumber in piles. It was in the investigation of the most important of these lumber stains in the study of the western yellow pine, *Pinus ponderosa*,† under Dr. Hermann von Schrenk of the Mississippi Valley Laboratory, of the United States Department of Agriculture, that the studies upon which this paper is based were begun. In addition to the blue stain in pine lumber, brown, black, pink, purple and yellow stains and blotches were noted, not only on pine boards, but also on gum, poplar and other kinds. These were found to be caused by a number of fungi, some of which were quite different from that which causes the blue stain of the western yellow pine, viz: *Ceratostomella pilifera* (Fr.) Winter. Again the bluing of wood was found to be the result of the action of more than one species of *Ceratostomella*.

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\* A thesis presented to the Faculty of Washington University, in candidacy for the degree of Doctor of Philosophy, April, 1906. Published by permission of the Secretary of Agriculture. — Type cultures of all of the species described as new in this paper have been divided into parts deposited respectively in the United States National Herbarium, the Physiological and Pathological Herbarium of the United States Department of Agriculture, and the Missouri Botanical Garden Herbarium.

† Von Schrenk, H. Bull. Pl. Ind. 36: 1-27. (1903).

The work of investigation has been undertaken primarily on account of the value of the knowledge which might accrue through the study of the chromogenic fungi or bacteria which are concerned in the color reactions which take place in stained lumber; yet, on the other hand, the economic value of the work has not been lost sight of, owing to the loss of hundreds of thousands of dollars to the lumber industry each year due to the lowering of the grade of lumber in piles through the rapid action of many tiny wood staining fungi.

In the study of the fungus flora of the lumber pile a large number of forms have been observed not all of which discolor the substratum upon which they grow. Attention has been given not only to those which penetrate wood deeply and stain it, but also to those which discolor it only superficially.

In the isolation and culture of the fungi herein described, recourse was had to the most careful bacteriological methods, such as are described by Dr. Erwin F. Smith\* in his excellent monograph on "Bacteria in relation to plant diseases." In a number of instances new conidial stages of fungi have been discovered, and in every instance the relation of the new form to the older known forms has been repeatedly established by starting with the newly discovered stage and following the fungus through all its known stages and back again to the beginning. The saprophytic nature of the forms studied made it possible to start with single colonies on a poured plate of agar medium and make transfer cultures to test tubes of sterile wood or other media. Each fungus was grown upon a number of kinds of wood, as well as upon potato, rice, bean, sweet potato, and other similar media in tubes, in addition to cultures upon agar media made from wood and other vegetable decoctions.

In order to better establish varietal and specific charac-

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\* Smith, E. F. Bacteria in relation to plant diseases. 1. (1905).

ters, parallel cultures of related fungi have been grown on sets of the same media in conditions as nearly identical as can obtain in an ordinarily well-equipped bacteriological laboratory. In order to make drawings of the delicate conidial stages of some species it was often found necessary to grow the colonies in Petri dishes of thin glass, and study them in the open in the dish, without placing a cover glass over the very fragile hyphae.

It is thought best to arrange the results under the following groups or heads: —

#### WOOD-STAINING FUNGI.

##### I. Wood-bluing fungi.

1. *Ceratostomella*.

##### II. Wood-blackening and wood-browning fungi.

1. *Graphium*.
2. *Hormodendron*.
3. *Hormiscium*.
4. Other wood-blackening fungi.

##### III. Wood-reddening fungi.

1. *Penicillium*.
2. *Fusarium*.

In conclusion of these introductory remarks, grateful mention should be made of the valuable assistance to the work of investigation accruing through the use of the library and other facilities of the Missouri Botanical Garden, kindly tendered by Dr. William Trelease, the Director. Acknowledgment also should be made of the helpful co-operation of Dr. Hermann von Schrenk, Mr. Perley Spaulding and Miss Laura L. Eames, of the Mississippi Valley Laboratory, and to Dr. A. D. Hopkins, of the Bureau of Entomology of the United States Department of Agriculture, for securing material for study and granting other similar favors.

#### I. WOOD-BLUIING FUNGI.

The blue stain in pine wood has been known in Europe for

many years. Hartig \* and Frank † both refer to it in their publications on plant diseases. Hartig ascribes the cause of bluing to *Ceratostoma piliferum* (Fr.) Fuckel, which is now placed under the genus *Ceratostomella* according to Winter. This species was first described by Fries, who placed it under the genus *Sphaeria*, where it remained with other species until Fuckel placed it under the genus *Ceratostoma*. Winter, ‡ in the revision of the genus *Ceratostoma*, placed all species with colorless ascospores under the new genus *Ceratostomella*.

Von Schrenk § in a bulletin of the Bureau of Plant Industry, U. S. Department of Agriculture, fully describes both the fungus *Ceratostomella pilifera* and its effect and mode of entrance into the wood of *Pinus ponderosa*.

A large number of species of *Ceratostomella* are described by Saccardo, ¶ many of which occur on wood, but no reference is made to the staining effect of any of these species. There is little doubt that a number of these are wood-staining fungi, but from the results of our investigations it appears that the common bluing fungus is *C. pilifera*.

### 1. CERATOSTOMELLA.

Winter in *Michelia* characterizes the genus *Ceratostomella* as follows: "Perithecia superficial or partly immersed, usually tough, leathery or carbonaceous, glabrous or invested with filaments, with prominent well developed beak; asci without paraphyses, 8-spored; ascospores continuous, globose, ovoid or oblong, hyaline; spermogonia and conidia present in some instances."

Although Winter mentions that conidia are present in a few instances, a careful search through the literature on the subject reveals that the number of species which have

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\* Hartig, R. *Lehrbuch der Pflanzenkrankheiten*. 75, 106. (1900).

† Frank, A. B. *Krankheiten der Pflanzen*. 1: 107. (1895).

‡ Saccardo, P. A. *Michelia*. 1: 370. § *l. c.*

¶ Saccardo, P. A. *Sylloge Fungorum*. 1-17.

conidia in their descriptions is very limited indeed, only two being noted, and where such descriptions have been given, no mention is made of the connection between the conidial and the perfect or perithecial stages as being established by cultural methods. For instance, *Ceratostomella albocoronata* (Ellis) Sacc. has a conidial stage where the conidia are from 2- to 3-septate, and of abnormally large size. So far as our investigation has proceeded, all species of *Ceratostomella* have colorless conidia, that are one-celled and of about the same measurements as the ascospores. The presence of asci in the perithecia has been the most difficult point to prove. Cultures must be taken at the stage just preceding full maturity of the ascus, else it apparently dissolves and frees the ascospores at maturity, allowing their free ejection through the long, narrow beak of the perithecium, but destroying the evidence of the presence of a sac. In an examination of the ejected ascospores they are frequently found adhering to each other in fours, side by side, in the same position they occupied in the ascus.

The conidial stage of *Ceratostomella* is very important, owing to the immense number of conidia borne on the mycelium in its earlier growth. These are readily disseminated by the wind and are probably carried by insects which penetrate the wood and bark of trees, like most of the ambrosia and bark beetles. At the stage in which the conidia form a mucilaginous mass, they adhere readily to any insect that may pass over them. In the laboratory a number of species of mites which feed on fungi carried spores on their bodies from a colony in an agar plate to a sterile portion of the surface of the medium and started new colonies of the fungus. Bark beetles were placed in a dish with the conidial stage of *Ceratostomella*, and after allowing them to remain a short time were transferred to sterile agar plates which were inoculated with spores from the insects. It is probable that some species of in-

sects feed on the conidial stage of *Ceratostomella*, especially one or more species of ambrosia beetles and a number of mites infesting their channels in the wood; but proof is yet lacking on this point. The constant occurrence of this fungus in the channels of a number of wood boring beetles indicates that the conidia or the ascospores must be carried in some manner by these insects. Hopkins\* describes some of these beetles in a bulletin of the U. S. Department of Agriculture, and von Schrenk† describes how *C. pilifera* follows the channels of wood-boring beetles in *Pinus ponderosa*. The writer has made similar observations on *Ceratostomella* in its penetration of *Pinus ponderosa*, *P. Arizonica*, *P. echinata* and *P. Virginiana*.

Although the genus *Ceratostoma* is very closely related to *Ceratostomella* none of the species of *Ceratostoma* have been found in connection with the blue stain of wood. In the course of the present investigation stained wood has been collected from a large number of localities, including wood from trees and shrubs of the following genera: *Abies*, *Acer*, *Fagus*, *Fraxinus*, *Liquidambar*, *Liriodendron*, *Pinus*, *Rubus*, *Ulmus*, *Vitis* and *Wistaria*. A number of species and varieties of *Ceratostomella* have been identified, cultivated and proved to be wood-bluening fungi. *Ceratostomella pilifera* has been found far more prevalent than other species. As the conidial stage of this species was first discovered by the writer and the description has never been published, it will now be given. It is taken from the results obtained from a very large number of pure cultures grown and studied over a period of nearly four years.

*Ceratostomella pilifera* (Fr.) Wint.

This fungus has been isolated in cultures taken from stained wood from a number of species of *Pinus* taken from nearly every region of the United States. It pene-

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\* Hopkins, A. D. Bull. Div. Ent. U. S. Dept. Agr. 32: 9, 10.

† Von Schrenk, H. l. c.

trates rapidly the sap wood of dead and dying trees, giving it a peculiar blue tint whose color is hard to explain, since the mycelium of the fungus in the cells of the wood is a dark brown. It has also been found in the sapwood of species of *Abies*, *Quercus*, *Fraxinus*, and other genera.

*Ceratostomella pilifera* grows readily upon a large number of culture media. Pine decoction agar made from the bark and sapwood was found an excellent medium for its cultivation. Two distinct fruiting forms were observed in all the varieties, the conidial and the perfect or perithecial.

#### MYCELIUM.

When either the conidia or ascospores are sown in pine agar plates, germination takes place in a few hours, and in two days colonies with a white hyaline mycelium develop. The filaments are septate, and, as a rule, branch alternately. In a day or so upright hyphae, either simple or branched, are sent out, upon which are borne branching whorls of conidia. In the course of a week or so, portions of the mycelium in the older region of the colonies develop with thicker walls and assume a brown color. From these dark colored filaments the perithecia originate.

The first growth of the mycelium is usually sparse, but under favorable conditions a secondary profuse growth with a fluffy white appearance develops, which bears conidia, and the formation of perithecia is retarded. Such a growth is formed on some of the richer media made from pine and other plant decoctions. Under these conditions the development of conidia is enormous, and the number of perithecia is decreased somewhat. Cultures on the sapwood of trees in test tubes bear both stages of the fungus abundantly. On the heartwood, however, but few conidia are formed, the mycelium is almost invisible to the eye, and perithecia are either sparse or entirely absent. The filaments of the fungus never penetrate deeply into



the tissues of heartwood of pine, oak, ash, or similar woods in artificial cultures, which fact coincides with the observation that in nature, for some reason, the same thing happens, the older discolored heartwood being usually free from attacks by the mycelium.

#### CONIDIA.

The conidia are borne on erect or-slanting hyphae which branch alternately from the mycelium. They develop terminally in branched or simple moniliform short chains which are often in whorls (pl. 4, f. 5). They are detached by the least movement, and in moist air fall together in agglutinated masses about the terminus of the hyphae, in a form which superficially resembles *Cephalosporium* (pl. 4, f. 6). These masses, however, are not so regular as the masses of conidia in the heads formed by *Cephalosporium*. The conidia bear some resemblance to those of *Ovularia*. The *Cephalosporium*-like clusters are always present in agar cultures after a few days' growth. The conidia vary from obovate to elliptical or cylindrical. They measure from  $8\mu$  to  $12\mu$  in length, and from  $2\mu$  to  $4\mu$  in width. They are hyaline when young, but often become vacuolate or guttulate when old. They are unicellular and borne on septate hyphae which are from  $3\mu$  to  $5\mu$  in diameter. Only hyaline filaments bear conidia. In germination the conidia send out terminal germ tubes from either end (pl. 4, f. 7).

#### PERITHECIA.

The first indication of the formation of perithecia on the mycelium is the union of two or more filaments and the formation of a knotted mass. The adjacent mycelium changes first to a light brown color, and later to a very dark brown. In the center of the mass the young perithecium develops first as a globular black body, without a beak or neck.

The walls of the perithecium are usually formed by one

layer of cells united in a rudimentary tissue. From the upper side of the young perithecium, after it has grown to about its normal size, there is projected a long tube or beak composed of parallel hyphae which terminate with colorless ends in a rounded tip. When the perithecium is mature it contains a large number of irregularly obovate or elliptical asci, each containing eight spores, usually arranged in fours. The beaked ostiolum, when the ascospores are ejected, splits at the end into a number of bristles which spread out and form a supporting crown for the ejected ascospores (pl. 3, f. 8). The perithecium without the neck measures  $160\mu$  in height and  $180\mu$  in diameter, average, and the neck about 1 mm. in height and  $20\mu$  in diameter, average. The ascospores average  $5.5\mu$  by  $2.5\mu$ .

It is quite evident that the sac containing the ascospores is dissolved at maturity, since it is rarely found with the mature ejected spores, but must be looked for in perithecia just approaching this stage. If the spores are ejected in open air they usually collect in a globular drop at the terminus of the neck, and when in this position the drop resembles very much the heads of some species of *Graphium* when covered with the mucus drop characteristic of the latter genus. If the spores are discharged in water they form worm-like sticky masses not readily miscible with water.

*Ceratostomella Schrenkiana* n. sp.

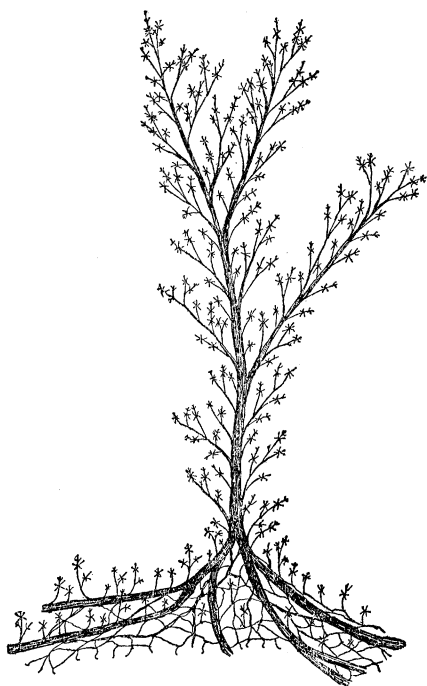
The sapwood of pine lumber made from *Pinus echinata* in Arkansas and other southern localities is stained by a number of fungi, among which are species of *Ceratostomella*, *Hormodendron*, *Graphium*, *Hormiscium* and *Penicillium*. A species of *Ceratostomella* which greatly resembles *C. pilifera* has been frequently collected on the wood of the southern pines by Dr. von Schrenk and others, from various localities in Missouri and Arkansas.

This species, when grown side by side with cultures of *C. pilifera* from the Black Hills pine, attains the same

size in so far as the colonies and perithecia are concerned; but on the other hand, both the conidia and ascospores are constantly much smaller. In nature, the perithecia that mature are often surrounded by several closely adhering dark bodies, which are either sclerotia or abortive perithecia, apparently the latter. This feature has never been noted with other species of *Ceratostomella*. In view of the differences, it is thought best to separate this form from *C. pilifera* and it is named *C. Schrenkiana*, in honor of Doctor von Schrenk, who first collected the fungus and noted its peculiarities.

The following cultural characters are taken from a large number of natural and artificial cultures: —

#### MYCELIUM.



*C. SCHRENKIANA*,  $\times 50$ .

Dendroid fruiting tuft.

Conidia or ascospores, if sown on agar plate cultures, germinate in a few hours, and after a day have formed conidia, if the temperature is favorable. The most favorable temperature for growth is  $80^{\circ}$  to  $90^{\circ}$  F. The filaments and hyphae remain white for several days, and often become massed in a furry outgrowth in which strands of filaments unite into upright clusters or false heads. (See figure). After a few days' growth, portions of the mycelium lying next the agar

become pigmented with a brown color, and rapidly develop

perithecia. The filaments of the mycelium measure  $3\mu$  to  $7\mu$  in diameter.

#### CONIDIA.

The conidia are borne in clusters similar to those of *C. pilifera* (pl. 4, f. 1, 2). They are unicellular, obovate to elliptical or cylindrical, and measure  $3\mu$  to  $7\mu$  by  $1\mu$  to  $2\mu$ , averaging  $5\mu$  by  $1.8\mu$ . They are hyaline and are usually neither guttulate nor granular, but when old may have one or two guttules.

#### PERITHECIA.

Perithecia are formed on the mycelium (pl. 4, f. 4), as in *C. pilifera*. The shape is spherical. They are coal black in color, and measure  $120\mu$  to  $200\mu$  in diameter, with a smooth, beaked ostium, measuring .8mm. to 1.2mm. in length by  $10\mu$  to  $25\mu$  in diameter, surmounted at maturity by a row of short, hyaline, spreading bristles, which support the ejected ascospores in a globular mass. The bristles measure about  $10\mu$  to  $15\mu$  by  $2\mu$  (pl. 3, f. 6). The ascospores are hyaline, pointed, elliptical, often slightly curved, and measure  $2.5\mu$  to  $4\mu$  by  $1\mu$  to  $1.5\mu$ , averaging  $3.5\mu$  by  $1\mu$  (pl. 4, f. 3).

This species resembles, in its gross measurements, the description of *C. echinella* E. & E. It differs, however, in that the perithecia are borne superficially, and are not glandular-pubescent or thickened at the tips. The mature perithecia are not gregarious, although they bear numerous small bodies at their base, under certain conditions.

#### *Ceratostomella echinella* E. & E.

The last species of *Ceratostomella* identified and studied before publication was collected by Dr. von Schrenk at Kirbyville, Texas, on the wood of the red beech, *Fagus atropurpurea* (Marsh.) Sudworth. This was growing on freshly cut heart and sap-wood, staining the wood either a bluish or

a brownish color. The fungus was isolated and grown in pure cultures on the wood of the red gum and on pine decoction agar, as well as on tubes of rice, potato, etc., the following cultural characters being from both natural and artificial cultures. The description differs but slightly from that given by Ellis and Everhart\* and has been emended by the addition of the conidial stage.

#### MYCELIUM.

The colonies resulting from either ascospores or conidia are white at first with a hyaline mycelium which later becomes pigmented in certain of the larger filaments, assuming a dark brown color. The filaments measure from 4 to  $7\mu$  in the mature forms. Conidia appear after 36 hours, and perithecia in four or five days.

#### CONIDIA.

The conidia are borne in short branching moniliform chains in small clusters, falling together in irregular masses as they mature (pl. 6, f. 1). They are unicellular, hyaline, becoming guttulate when old, obovate to elliptical, and measure from  $4\mu$  to  $6.5\mu$  by  $2\mu$  to  $3.5\mu$ , averaging  $6\mu$  by  $3\mu$ .

#### PERITHECIA.

The perithecia are brown at first, but when they are mature they become black and carbonaceous and are covered sparsely with short, glandular hairs. They are globose, or sometimes slightly flattened, measuring  $50\mu$  to  $100\mu$  in diameter. The hairs measure  $10\mu$  to  $32\mu$  in length by  $1.5\mu$  to  $2\mu$ . Each terminates in a gland or spherical enlargement measuring  $2\mu$  to  $3\mu$  in diameter. The perithecium is tipped by a long slender beak, black at the base, brown

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\* Ellis, J. B. and Everhart, B. M. North American Pyrenomycetes. p. 195. (1892).

or gray at the apex, but smooth throughout measuring 1 mm. to 1.7 mm. in length by  $15\mu$  to  $25\mu$  in diameter and surmounted when mature by a terminal fringe of bristles  $15\mu$  to  $25\mu$  in length and tapering from  $4\mu$  at the base to  $1\mu$  at the apex, being hyaline throughout (pl. 3, f. 3). The asci are clavate(?), bearing biseriate spores which are hyaline, curved or straight, elliptic to cylindrical, and which are ejected in the open air in a globular mass at the terminus of the beak.

Since writing the above, co-type material of *C. echinella* in the herbarium of the Missouri Botanical Garden has been examined. The perithecia are probably as described by Ellis and Everhart,\* but they have persistent asci which contain brown two-celled ascospores, placing the fungus in the specimen under another genus. This indicates that at least a mistake was made in a portion of the material sent out as type specimens if not in the description.

*Ceratostomella capillifera* n. sp.

The wood of *Liquidambar styraciflua* L. is an excellent pabulum for a large number of fungi, and lumber made from this tree, when in piles, soon becomes thoroughly stained either blue or black by the growth of a number of species of wood-discoloring fungi. Among these was found quite commonly a species of *Ceratostomella* which is closely related to *C. pilifera*, but differs in the length of the beaked ostiolum, the length of the terminal bristles and the shape and size of the conidia and ascospores. The following characters obtain with pure cultures: —

MYCELIUM.

Cultures of either conidia or ascospores sown on pine-decoction agar germinate quickly, forming a diffuse, white, hyaline, septate mycelium, which in less than two days

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\* Ellis, J. B. and Everhart, B. M. *l. c.*

bears hyaline conidia abundantly. In about a week, black, long-beaked pycnidia appear on certain portions of the mycelium, which assumes a brown color, the development being similar to that described for *C. pilifera*. The filaments of the hyaline portion of the mycelium measure from  $2\mu$  to  $3\mu$  in diameter, and the older brown-colored filaments, in wood, from  $3\mu$  to  $6\mu$ .

#### CONIDIA.

The conidia are borne like those of *C. pluriannulata*, and are similar in shape (pl. 6, f. 2, 3). They are unicellular, hyaline, elliptical to cylindrical, and measure  $4\mu$  to  $8\mu$  in length, and  $1.5\mu$  to  $2\mu$  in width averaging  $6\mu$  by  $1.8\mu$ .

#### PERITHECIA.

The perithecia are spherical, of a black color, with black, long-beaked ostiola, terminating in long, wavy hyaline filaments. The body of the perithecium is usually nearly covered with dark brown hyphae or filaments. It averages  $200\mu$  in diameter, and the neck  $1.5$  mm. in length and  $25\mu$  in width. The terminal filaments are long and slender and measure  $80\mu$  by  $1\mu$ , average (pl. 3, f. 1).

The ascospores are elliptical to reniform in shape and average  $4.5\mu$  by  $1.5\mu$  in size. It will be noted that although the perithecia of this species are larger than those of *C. pilifera* the conidia are somewhat smaller. Both ascospores and conidia usually germinate terminally, some few exceptions having been noted.

The name *C. capillifera* is given to this species on account of the long minute bristles which project from the ostiolum.

#### *Ceratostomella pluriannulata* n. sp.

A block of blued sapwood from *Quercus rubra* was collected in 1905 by Perley Spaulding and turned over to the

author for investigation. A species of *Ceratostomella* was found among the cells of the wood and fruiting quite abundantly on the surface. The characters of the fungus agree in part with *C. pilifera*, to which it is closely related. It has ascospores which are constantly of a slightly different shape, and is often characterized by more than one whorl of bristles on the beaked ostiolum. The conidia are considerably smaller, and the hyphae on which they are borne are more frequently branched, thus giving rise to larger clusters. Owing to the frequent presence of more than one ring of bristles on the ostiolum, the name *C. pluriannulata* is here given to the species, with the following cultural characters: —

#### MYCELIUM.

The growth of the mycelium from either conidia or ascospores resembles that of *C. pilifera*. The spores germinate in pine-decoction agar in 2 to 3 hours, and in 2 to 3 days a white, floccose mycelium bearing conidia appears. In from 5 to 10 days perithecia appear, the development of which is like that of *C. pilifera*.

#### CONIDIA.

The hyaline unicellular conidia are borne on branching septate, hyaline, upright hyphae in simple or compound whorls (pl. 5, f. 1). In the older portions of the colony, the conidia fall off and adhere in masses forming clusters resembling *Cephalosporium*. These clusters vary in size from  $10\mu$  to  $100\mu$  in diameter. The larger clusters are formed by the adherence of several spore clusters into one mass. The conidia measure  $5\mu$  to  $8\mu$  in length and  $2\mu$  to  $3\mu$  in width, averaging  $6\mu$  by  $1.5\mu$ .

#### PERITHECIA.

The perithecia are borne superficially on the wood, or on the mycelium, there being no definite stroma in this or in



the other species of *Ceratostomella* studied. The perithecia are globose, varying from  $90\mu$  to  $200\mu$  in diameter, and averaging  $120\mu$ . The lengthened beak measures from .9 to 2 mm. in length, and  $10\mu$  to  $30\mu$  in diameter, averaging 1.5 mm. by  $25\mu$ . Some of the necks, in addition to the ring of bristles ordinarily found at the terminus of the beaked ostiolum, have one or two whorls at some distance below the end (pl. 3, f. 7). This character has never been noted in cultures of *C. pilifera*. The terminal bristles are tapering and average  $20\mu$  in length by  $2\mu$  in width. The asci have not been measured. The ascospores are reniform in shape, hyaline, and measure  $4\mu$  to  $5\mu$  in length, and  $1.5\mu$  to  $1.7\mu$  in width, averaging  $4.5\mu$  by  $1.5\mu$  (pl. 5, f. 2).

This species penetrates pine wood when inoculations are made, nearly as readily as *C. pilifera*. The mycelium in the wood cells follows the medullary rays in pine wood, but in red gum wood it penetrates also numerous tracheary vessels, and sometimes the wood fibres. It is most abundant, however, in the medullary rays of the latter wood.

*Ceratostomella minor*, n. sp.

During the year 1905 some specimens of pine wood stained with a dark blue color, from *Pinus Arizonica* Eng., were received from Dr. A. D. Hopkins. From these there were isolated two species of wood-staining fungi; one a species of *Ceratostomella*, the other a *Graphium*. The *Ceratostomella* proved to be smaller than the species from *Pinus Virginiana*, and differed in other points. The fungus gained entrance through the galleries of wood beetles, the stain radiating from cavities made by these insects. When grown in pure culture the fungus possessed conidia and ascospores. As the specific characters do not agree with any previous description of *Ceratostomella*, the name

*C. minor* is assigned, with the following cultural characters: —

#### MYCELIUM.

Cultures of either conidia or ascospores on pine agar media germinated in a few hours, and in two days the colonies began to form conidia. The mycelium is white and sparse. The hyaline filaments of the conidial stage measure in width  $1.5\mu$  to  $2.6\mu$ , averaging  $2.3\mu$ . The brown or black filaments in agar cultures and in wood measure in diameter from  $2\mu$  to  $4\mu$ , averaging  $3.5\mu$ . These are often rugose, the roughenings being coarser than those in *C. exigua*. In some portions of the mycelium on wood there are filaments that contain cells that are unequal in diameter, being enlarged at one end. Like *C. pilifera* this species follows chiefly the medullary rays of pine wood.

#### CONIDIA.

The formation of the conidia and the form of fruiting do not differ essentially from those of *C. pilifera* and *C. exigua*. The unicellular, hyaline conidia measure from  $4\mu$  to  $5.5\mu$  in length, and from  $1.8\mu$  to  $2\mu$  in width, averaging  $4.5\mu$  by  $2\mu$ . They are oval to elliptical in shape, and fall off at the slightest touch, collecting in rounded masses on the hyphae when in moist air.

#### PERITHECIA.

The perithecia mature in about three weeks in artificial cultures. They are spherical, black rugose bodies, varying from  $40\mu$  to  $70\mu$  in diameter, averaging  $52\mu$ , with a beaked ostium  $120\mu$  to  $160\mu$  in length, averaging  $135\mu$ , and from  $6\mu$  to  $12\mu$  in diameter (pl. 5, f. 6). The beak ends in a whorl of short, thick bristles (pl. 3, f. 4). The asci are round to oval, hyaline, with eight spores in each, usually arranged in fours (pl. 5, f. 7). The spores measure  $3.1\mu$  to  $4.2\mu$  in length and  $.9\mu$  to  $1.9\mu$  in width, averag-

ing  $3.5\mu$  by  $1.5\mu$ . They are hyaline and germinate terminally.

The perithecia are smaller than those of *C. exigua*, the description of which follows; the ascospores differ in shape, and are smaller, and the terminal bristles of the beak have a different shape, being short and thick as compared with the slender ones of *C. exigua*.

*Ceratostomella exigua* n. sp.

The wood of dead and dying trees of *Pinus Virginiana* Mill., and one or two other species, is often colored a blue-black color, even more intense than that produced by *Ceratostomella pilifera* in wood of the western yellow pine. Specimens were first obtained for study from Dr. A. D. Hopkins of the United States Department of Agriculture. It was at first thought that the stain was caused by *C. pilifera*, but pure cultures of the fungus proved that it was different in a number of particulars. The scanty mycelium, the numerous diminutive perithecia and the smaller conidia, as well as the more intense color of the stain, were prominent points of difference which were considered of specific importance. These characteristics remained quite constant through a series of cultures on various kinds of wood.

The life history of the fungus obtained from a series of pure cultures is as follows: —

MYCELIUM.

Cultures grown on pine decoction agar from both conidia and ascospores give in a short time colonies with a white mycelium much more limited in growth than that of *C. pilifera*. The conidial stage appears after 24 hours, and is of the same type as that of *C. pilifera*, but with the conidia in lesser numbers. In a few days the perithecia appear on a mycelium which assumes a very dark brown,

almost black, color. The development of the fungus on wood is slower than that of *C. pilifera*, staining pine, oak, elm and ash with a blue-black discoloration contrasting sharply with the unstained portions. The mycelium penetrates pine wood through the medullary rays, and in some cases enters the wood fibres. The hyphae of the hyaline mycelium bearing conidia average  $2.5\mu$  in diameter, while the dark brown filaments in wood measure from  $2\mu$  to  $6\mu$ , averaging  $4\mu$  in diameter. On the surface of wood in the vicinity of perithecia some filaments have swollen cells. Many of the filaments in wood cells have fine transparent roughenings all over the surface.

#### CONIDIA.

The conidia, like those of *C. pilifera*, are borne in whorls of single spores and short moniliform chains intermingled (pl. 6, f. 4, 5). In older cultures they drop together in clusters resembling somewhat those of *Cephalosporium*. They are oval to elliptical in shape, one-celled, hyaline, and vary in size from  $3.5\mu$  to  $4.5\mu$ , in length, and  $1.6\mu$  to  $2.2\mu$  in width, averaging  $4\mu$  by  $2\mu$  (pl. 6, f. 6).

#### PERITHECIA.

In artificial as well as in natural growths, the perithecia are very abundant. They are formed in large numbers under favorable conditions after 10 to 14 days. The process of formation is similar to that of *C. pilifera*. The perithecia are globose. They are black, brittle, with a roughened surface. They measure  $60\mu$  to  $80\mu$ , averaging  $73\mu$  in diameter, with a beaked ostium  $150\mu$  to  $200\mu$  in length, and  $8\mu$  to  $18\mu$  in diameter, averaging  $180\mu$  by  $14\mu$  (pl. 3, f. 2). The asci are hyaline, irregularly oval to elliptical, and contain 8 spores, often arranged in fours. The ascospores are hyaline, elliptical, sometimes slightly curved, and measure  $2.1\mu$  to  $2.8\mu$  in length, and  $.8\mu$  to  $1.1\mu$  in width, averaging  $2.5\mu$  by  $1\mu$  (pl. 6, f. 7). The sacs usually disappear before the spores are ejected.

This species resembles the description of *C. microspora* E. and E. but differs in the shape of the ascospores, the size of the perithecia, and the length of the beaks. It has, in addition, a conidial stage, which serves to further set it apart as a new species. On account of the minuteness of its perithecia and spores it is named *C. exigua*.

*Ceratostomella moniliformis* n. sp.

Another species of *Ceratostomella* was found by Dr. von Schrenk growing on gum wood in Texas, near Kirbyville, occurring on *Liquidambar styraciflua*. This species stains the wood of gum a brown color, and is the most rapid growing form studied. It is distinct from the other species of *Ceratostomella* in the form of the conidial clusters, and in the early color of the mycelium. The perithecia are covered with short spines, otherwise resembling very much those of *C. pilifera*. The name *Ceratostomella moniliformis* is given to the fungus, with the following cultural characters:—

MYCELIUM.

Cultures of either conidia or ascospores sown in agar plates germinate quickly, and in 24 hours produce the conidial stage. In two or three days the perithecia are produced. The colony is hyaline at first, but in a few hours begins to turn gray, and finally becomes black. The mycelium is coarsely granular, the filaments measuring  $2\mu$  to  $8\mu$  in diameter.

CONIDIA.

The conidia are found on simple or branching, upright hyphae, both in simple moniliform chains and in terminal clusters, the latter resembling the form occurring with some species of *Graphium* (pl. 5, f. 3, 4, 5). They are formed by the abstriction of the ends of the hyphae, and often fall together as fast as they mature, in rounded

masses, surrounded by either water or mucilage. They are unicellular, cylindrical in shape, hyaline, and measure  $6\mu$  to  $8\mu$  by  $1.8\mu$  to  $2.2\mu$ , being of different shape and dimensions from the ascospores, and are thus easily distinguished from them under the microscope. The conidia in mass as they become old have a gray color.

#### PERITHECIA.

The perithecia are formed by the union of two or more filaments of the mycelium. First, a gray mass of irregular cells appears at the point of union. This rapidly develops into a globular perithecium, from the top of which there is then rapidly thrown out a beaked ostiolum, consisting of parallel filaments. The perithecia are brown to black in color,  $90\mu$  to  $180\mu$  in diameter, covered with sparse, conical spines,  $12\mu$  to  $16\mu$  long, with a diameter of  $6\mu$  at the base. The beaked ostiolum is brown to black throughout its length, striate, and is surmounted by short, rather thick, hyaline bristles, measuring  $12\mu$  to  $18\mu$  by  $2\mu$  (pl. 3, f. 5). When the perithecia are mature, the ascospores are ejected in water in long, slimy, gray masses. In nature they are ejected in an irregular gray mass supported by the terminal filaments of the beak. The asci are fugacious, hyaline, oval, and average  $20\mu$  by  $10\mu$ . The ascospores are hyaline, not guttulate, at least at first, oval, often flattened on one side, measuring  $4\mu$  to  $5\mu$  by  $3\mu$  to  $4\mu$ .

This species of *Ceratostomella* is so different in color from the other species studied as to suggest that it may be a *Ceratostoma*. The gray color of the mycelium on media in which the mycelium of other species remains hyaline, the constant tint of gray in mature conidia and ascospores, and the peculiar moniliform chains of conidia very distinctly set this form apart at least as a very distinct species. But since the color of the spores shows only in mass, it is

thought best to place the species at present under *Ceratostomella*.

## II. WOOD-BLACKENING AND WOOD-BROWNING FUNGI.

### 1. GRAPHIUM.

*Graphium* has been associated with the decay of wood and other organic matter ever since the original species were named by Tode\* and Corda,† and placed under *Stilbum* by Corda. It has been found also to be associated with the staining of lumber, especially of pine and gum boards. It has been isolated from black or brown stained portions of wood taken from *Pinus*, *Populus*, *Liriodendron*, *Liquidambar*, *Quercus*, *Acer* and *Wistaria*. Species of this genus, like those of *Ceratostomella*, enter most kinds of wood through the medullary rays, being usually confined to the sapwood, with the exception that they may grow upon decayed heartwood that other fungi have previously attacked. In the study of a number of species of *Graphium* not all species stained wood alike: some scarcely stained the wood at all, others, like the one from *Liquidambar*, are quite effective as wood stainers.

The stain given by *Graphium* varies from a dirty gray to a dark brown, or, rarely, a black color.

The genus *Graphium* was first described by Corda. It is described as follows, using a free translation: — “Stroma cylindrical, clavate, or capitate, brownish, rather rigid, the upper hyphae paler, lax, and bearing the conidia, which are elliptical or oblong, hyaline, often involved in mucus at first.” No mention is made either by Saccardo,‡ or Engler and Prantl§ of any conidial stage other than that found on the stroma or head.

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\* Tode. Fungi Meckl. 1: 10. † Corda. Icon. Fung. 1: 8.

‡ Saccardo, P. A. Michelia. 2: 32; Syll. Fung. 4: 564, 609.

§ Engler, A. and Prantl, K. Die Natürlichen Pflanzenfamilien. 1: 493.

Boulanger\* has proven by the use of careful cultural methods that *Sporotrichum chlorinum* Link var. *grisea* Boul. is a stage of *Graphium eumorphum* Sacc. This discovery is now verified by the present investigation, and *Sporotrichum*-like stages of other species of *Graphium* are now published for the first time. Boulanger also discovered a *Sporotrichum* stage of *Chaetomium cuniculorum*, and obtained fruits of the latter fungus in connection with *Graphium*, indicating that *Chaetomium cuniculorum* is the perfect stage of the other two fungi. We have not been able to verify the latter relationship.

*Graphium giganteum* (Pk.) Sacc. is given by Durand† as a conidial stage of *Holwaya gigantea* (Pk.) Durand.

Zopf‡ observed a moniliform conidial stage of *Chaetomium* which failed to germinate. He makes no mention of finding any related forms of *Sporotrichum*.

Some of the species we have studied contain both *Sporotrichum* forms and moniliform fruits, the latter resembling very much the branching clusters of the conidial stage of *Ceratostomella*.

From the study of several species of *Graphium* the following cultural characters are found to be common:—

#### MYCELIUM.

The mycelium in a culture from either of the two spore-forms found in the species studied is from the first hyaline and septate. On rich vegetable-decoction agar, like that made from green pine sapwood, bean pods, potato, etc., a fluffy white mycelium is usually produced, varying in density with the medium and the species. The first form of conidia is borne on erect hyphae, which may be either simple or branched. These hyphae or conidiophores

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\* Boulanger. Rev. Gén. de Bot. 1895: 97-102.

† Durand, E. J. Bull. Torr. Bot. Club. 28: 351. (1901).

‡ Zopf, W. Ueber Pilzfarbstoffe. III. Bot. Zeit. 47: 86-89. (1889).



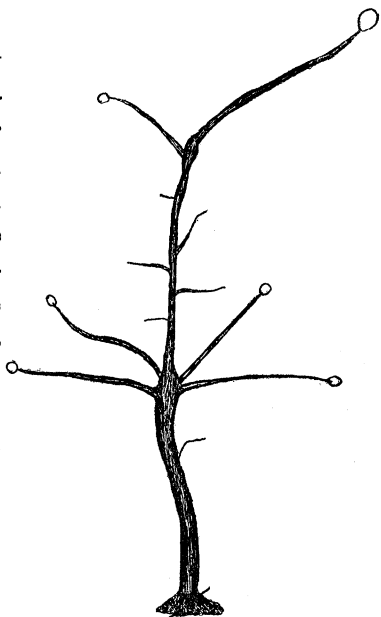
branch alternately from the mycelium. These first conidia are borne in clusters of three types, two of which are simple clusters of the type of those of *Sporotrichum* but not borne on prostrate hyphae, the other being a form which is like most of the conidia described under *Ceratostomella*. In this latter form the conidia are borne in the short branching chains. These conidia are all ephemeral and do not hold their vitality as long as those which are produced later upon the heads or stromata. In order to distinguish them from the latter, they are now for the first time called secondary conidia; and those of the heads or stromata are now called primary conidia. As the colony grows older, some portions of the mycelium become more or less pigmented, and the darker filaments grow together in masses from which erect columns of parallel hyphae spring forth. These bear heads composed of branched hyphae, which produce the primary conidia terminally by abstriction. As fast as the conidia are formed they drop away into a mucous matrix which forms over the surface of the head.

#### SECONDARY CONIDIA.

The secondary conidia of the *Sporotrichum*-type are of two forms and are borne on simple erect or branched hyphae. The first form of this type are attached to the hyphae in spreading, open clusters, which do not fall together in masses until some days after they mature (pl. 7, f. 3). The second form are produced in close clusters which fall together as rapidly as they mature (pl. 9, f. 9). The third form of fruiting, as mentioned in the previous topic, more nearly resembles the branching chains of the conidia of many species of *Ceratostomella* (pl. 9, f. 3). The spores in all three forms are oval, elliptical, or cylindrical, and correspond closely in form and measurement to the primary conidia of the same species. They are unicellular and formed by abstriction either from the terminus of the hyphae or from short, terminal or lateral branches.

## PRIMARY CONIDIA.

After the primary conidia have been detached and separated from the mucus there is in many species nothing to distinguish them from the secondary conidia. In fact, the real difference usually in the two forms is whether they are borne on a single hypha, or on hyphae united in a stroma. The agglutinated cluster on a single stalk of one hypha corresponds morphologically to the mucous-covered head (pl. 7, f. 2, 5). The latter are more persistent, and retain the conidia for a much longer time. A secondary growth of the stromata has been noted in some species after the first spores have been formed, and have dropped away, in which there are formed branches from the end of an old stalk, growing out from the apex. (See figure). Each of these subsidiary stalks is composed of a number of parallel filaments, or hyphae, which in turn branch at the outer ends and form masses of conidia like the original head, only smaller. Such growths take place on rich, starchy media like cooked potatoes. On cultures on rice and potato an abortive type of stromata is often formed in the shape of somewhat flat, branching stalks, which grow much taller than the fruiting form, but bear no conidia. This abortive form resembles very much the original description of the sterile fungus *Anthina* Fr., which is probably a form of *Graphium*.

G. EUMORPHUM,  $\times 50$ .

Proliferation of stroma.

In the gross appearance of artificial cultures of *Graphium* and of *Ceratostomella* there is often a striking resemblance or mimicry. The secondary conidial stage of the former resembles superficially the conidial stage of the latter, and the mucous head of the former that of the perithecial stage of the latter at the time the ascospores are ejected in a viscid drop. The mucus surrounding the conidia of *Graphium* is soluble in water; on the other hand, the jelly-like substance exuded from the perithecia of *Ceratostomella* is very difficultly soluble in water. Both are well adapted for adhering to the bodies of insects with which they come in contact.

#### PERFECT OR CHAETOMIUM STAGE.

The *Chaetomium* stage of *Graphium* observed by Boulanger has not been found in connection with the species studied; but as the cultural work on *Graphium* has not yet been concluded, it is hoped that by the use of the proper culture media some species may finally be stimulated to produce perfect forms, either of *Chaetomium* or other genera.

In the consideration of the species of *Graphium* studied, it is thought best to separate them into the following groups based on the form of fruiting in the secondary conidial stage: —

- A. Species of *Graphium* with a secondary conidial stage with conidia resembling those of *Sporotrichum*.
- B. Species of *Graphium* with a secondary conidial stage unlike *Sporotrichum*.
  - 1. Species with secondary conidia borne continuously and terminally, falling at once in clusters.
  - 2. Species with secondary conidia borne either in simple clusters or in clusters of short branched chains.

A. SPECIES OF *GRAPHIUM* WITH A SECONDARY CONIDIAL STAGE WITH CONIDIA RESEMBLING THOSE OF *SPOROTRICHUM*.

*Graphium ambrosiigerum* n. sp.

A species of *Graphium* was found growing in the galleries of wood-boring beetles in the wood of *Pinus Arizonica* Eng. This material was furnished by Dr. A. D. Hopkins in connection with the study of the relation between ambrosia fungi and ambrosia beetles, a subject which is still under consideration in connection with the study of such beetles by Dr. Hopkins. Pieces were taken from the stained wood in the interior of the block near a gallery, and placed in test tubes of pine sapwood, which had been sterilized after being moistened. From the mycelium in the stained wood there grew out a white growth, bearing at first the secondary then the primary conidia of *Graphium*. Secondary conidia were found in some of the galleries when the material was first examined, but were not recognized as such at the time. The following are the cultural characters of the fungus:—

MYCELIUM AND SECONDARY CONIDIA.

When cultures are made with either the primary or secondary conidia on pine decoction agar, the spores generally germinate in a few hours, and in two or more days colonies may be noted with a white, sparse mycelium. At this stage of growth, secondary conidia appear of the *Sporotrichum*-type, borne on erect, simple hyphae (pl. 8, f. 5). These are hyaline, and oval in shape. After a few days, portions of the mycelium assume a brownish color, and from certain matted masses of filaments or stromata there grow out tufts of erect, parallel hyphae (pl. 8, f. 6).

These tufts of hyphae elongate into a long, round stalk, terminating in a bunch of either verticillate or alternate branches.

#### PRIMARY CONIDIA.

The primary conidia are formed on the upper portion of the stroma or head by an abstriction of the ends of the hyphae (pl. 8, f. 7). They are formed continuously, and as soon as mature, fall away into the mucous mass which surrounds the head. The mass is hyaline at first, changing, as it grows older, to a straw color, and finally to a dark brown. The stalk is usually hyaline in the first stages of its growth, but later becomes almost black at the base, shading off to a light brown near the apex, with a striated appearance. No chlamydospores or sclerotia are formed. At first the stroma is simple, but by the addition of branches from the base becomes gregarious (pl. 8, f. 4).

The following are the measurements of the mycelial structures: the hyaline mycelium measures from  $1.5\mu$  to  $2.5\mu$  in diameter, averaging  $2\mu$ . The colored portion averages in diameter about  $4\mu$ . The secondary conidia measure  $3.5\mu$  to  $4\mu$  in length, and  $1.2\mu$  to  $1.5\mu$  in width, averaging  $3.7\mu$  by  $1.3\mu$ . The primary conidia average  $5\mu$  by  $3\mu$ . The stalks average in length from  $500\mu$  to  $900\mu$ . The head, including the mucous drop, measures on an average  $200\mu$ . These do not agree with any previous description of species of *Graphium* and the fungus is here named *Graphium ambrosiigerum*, owing to the relation of the conidial stage to ambrosia beetles.

Cultures were made on a number of kinds of wood. The fungus grew readily in the sapwood of pine, gum, oak and ash, bearing in most cases numerous heads. Secondary conidia are usually formed in abundance. Cultures on the heartwood of these trees produce little or no growth of the fungus. Light colored wood is stained a color varying from a dirty gray to a light brown, or almost black.

The mycelium penetrates pine and other similar woods through the medullary rays, dissolving the starch. In gum wood, *Liquidambar styraciflua* Linn., it penetrates in addition many of the vessels of the wood. As compared with *Ceratosomella* the penetration is not so rapid, neither is the stain so intense; but on the other hand it may in time stain wood as deeply.

*Graphium eumorphum* Sacc.

A species of *Graphium* has been found frequenting the old wood of *Rubus strigosus* and related species. This was isolated and grown first on wood of several kinds which it was found to stain lightly, then upon agar media. An examination of the cultural characters of the fungus place it readily under *Graphium eumorphum* Sacc. The following description gives the principal characters, from both natural and artificial cultures:—

MYCELIUM.

Cultures made from both the primary and the secondary conidia produce a sparse, white mycelium, which soon turns a gray-green, and whose filaments measure from  $1\mu$  to  $2\mu$  in diameter. These soon bear numerous conidia of the *Sporotrichum*-type (pl. 7, f. 2), and about a week later the stromata or stalked heads of *Graphium* appear (pl. 7, f. 1). The heads are white, changing to a green or greenish yellow, and measure with the mucilage drop from  $30\mu$  to  $100\mu$ . The stalks vary from a yellow to a dark olive or a brown color, and measure from  $300\mu$  to  $500\mu$  in length, and from  $10\mu$  to  $40\mu$  in diameter.

SECONDARY CONIDIA.

The secondary conidia are obovate to elliptical in shape, and average  $7.8\mu$  by  $3.4\mu$  (pl. 7, f. 3). They are borne,

unlike *Sporotrichum*, on simple erect not prostrate hyphae. This stage nevertheless is apparently identical with *Sporotrichum vellereum* Sacc. and Speg. var. *grisea* Boul.

#### PRIMARY CONIDIA.

The primary conidia are borne on branching hyphae in stalked heads (pl. 7, f. 5), and average  $7.7\mu$  by  $3.4\mu$ . They are of a light green or yellow tint, and are identical in shape and appearance with the secondary conidia.

#### *Graphium atrovirens* n. sp.

In addition to *Graphium smaragdinum* on the red gum wood a new species was found present in a number of instances. This discolored the wood with a brown stain, similar to the latter species, and at first was not distinguished from it, having the same color and appearance in mass; but after a more careful study of the colonies on agar, different forms of secondary conidia were found present in two types of colonies, making it necessary to separate this form from *G. smaragdinum* and giving good reasons to call it a different species. It is now named *Graphium atrovirens* with the following as its cultural characters:—

#### MYCELIUM.

The growth of the colonies on agar plates is fluffy, as compared with that of *G. smaragdinum*. This is due to the formation of strands in the mycelium by the union of a number of parallel filaments, these growing upward and often forming large branching masses, similar to those formed by the mycelium of some species of *Cerastostomella*. The filaments of the mycelium are hyaline at first, later changing to dark green or olive, in the vicinity of the stromata or heads. They measure  $3\mu$  to  $4\mu$  in diameter. Those which unite to form the stroma usually measure but

$2\mu$  in diameter. The color of the stroma is dark green to black when mature, but in growing colonies all variations between hyaline and dark green may be found. The stalks measure from 1.5 mm. to 3 mm. in height, and  $8\mu$  to  $80\mu$  in diameter, being proportionately more slender than those of every other species of *Graphium* described in this paper (pl. 8, f. 1). In this species, as in others, all the forms of gradation between a head with a stalk composed of one filament, and a stalk with many filaments may be found. The difference between those bearing secondary conidia and those bearing primary conidia consists mainly in the absence of color in hyphae bearing the former.

#### SECONDARY CONIDIA.

The secondary conidia are borne in simple open clusters, of the type of the conidia of *Sporotrichum*. They are borne either terminally or along the sides of lengthened hyphae (pl. 8, f. 2, 3). They are obovate to elliptical in shape, measuring  $4\mu$  to  $5.5\mu$  by  $1.6\mu$  to  $2\mu$ . They are hyaline, becoming guttulate when old. The clusters remain open for a number of days after they are mature, but under very moist conditions fall together in rounded masses on the hyphae. The conidia of *G. smaragdinum* fall together as soon as mature, the clusters being much closer in their formation (pl. 9, f. 9).

#### PRIMARY CONIDIA.

The primary conidia are borne terminally on the ends of the branching hyphae in the stroma which form large, mucilaginous heads. These, without the sheath of mucus, are flattened oval in shape, (pl. 8, f. 1), white at first, later gray in color. They measure  $40\mu$  to  $600\mu$  in their greatest diameter, the latter being the measure of the head including the mucilaginous sheath. The primary conidia are hyaline, obovate to elliptical, and measure



$3.5\mu$  to  $4.5\mu$  by  $1.4\mu$  to  $2\mu$ . The stalks in this species bearing the primary conidia are sometimes much branched, but this feature is dependent more upon the culture medium and physical conditions than upon the inherent specific tendencies. One difference between the stalks of this species and those of *G. smaragdinum* is that in the case of the latter they tend to be gregarious, and are more apt to be swollen at the base.

B. SPECIES OF *GRAPHIUM* WITH A SECONDARY CONIDIAL STAGE UNLIKE *SPOROTRICHUM*.

1. Species with secondary conidia borne continuously and terminally, falling at once into clusters.

Under this subdivision will fall at least two species, *Graphium smaragdinum* and *G. rigidum*. A third species is still under culture but the work has not progressed sufficiently for identification.

It is a very difficult matter to determine the manner in which the secondary conidia are borne in these species, because as rapidly as the conidia are formed by abstriction from the end of simple or branched hyphae they break off and cling closely to the ends of the hyphae, being enveloped in a thin coating of water or soluble mucilage which hides the outline of the hyphae and conidia. It is probable that there is sometimes more than one conidium attached directly to the end of each hyphae branch and that these secondary conidia are but modified forms of those described in division A. The conidial masses formed are similar in appearance to those of *G. ambrosiigerum*, *G. eumorphum* and *G. atrovirens*.

It is also quite probable that in all species of *Graphium* the primary conidia are formed in the head precisely as the secondary conidia are formed in the open and that the two

forms of conidia are morphological equivalents. In most of the species studied many gradations were found between a head on a stalk of a single colored filament and heads on stalks of two to many filaments.

*Graphium smaragdinum* (A. & S.) Sacc.

The wood of the red gum, *Liquidambar Styraciflua* L., when freshly sawn and piled up, is very rapidly stained by the combined effects of a number of fungi. The most important among these are *Ceratostomella*, *Graphium*, *Homodendron*, and *Dematium*. On the surface, especially on the sapwood, the fruits of *Graphium* appear more frequently than those of the other fungi, especially when the boards are placed in a moist chamber. *Graphium* was obtained from gum boards taken from a number of localities, and in most instances a species was found which resembled *Graphium rigidum* but differed from it in a number of its cultural characters. From the following characters, taken from both natural and artificial cultures, it corresponds more nearly to *Graphium smaragdinum* (A. & S.) Sacc., and it is assigned to this species.

MYCELIUM.

Primary and secondary conidia, sown in agar plate cultures, germinate in a few hours, and small, white colonies are visible in a day. The mycelium is abundant, floccose; the filaments are septate, hyaline at first, later changing to dark green, measuring from  $2\mu$  to  $4\mu$  in diameter. In two days secondary conidia appear, borne terminally on simple hyphae, (pl. 9, f. 9). In less than a week, on dark colored portions of the mycelium, the stalks and heads or stromata of the *Graphium* stage appear (pl. 9, f. 8). These are hyaline at first, changing as they age to dark green, then to black at the base, shading off to a light gray-green

at the top. The stalks vary in height from 1 to 2 mm., averaging 1.5 mm., with an average diameter of  $25\mu$ . The heads, when enveloped in mucus, are globular and measure from 40 to  $600\mu$  in diameter; without the sheaths they are flattened oval or fungiform, and measure  $30\mu$  to  $200\mu$ . They are colorless at first, but later change to gray or green. When a stalk is broken off before maturity, it often sends out small branches, either of single hyphae or of clusters of hyphae, bearing conidia.

#### SECONDARY CONIDIA.

The secondary conidia are usually elliptical in shape, and measure  $3.6\mu$  by  $1.8\mu$ , average. They are hyaline, colorless, and are borne both terminally in close clusters and on simple hyphae, and in a short time fall off and adhere together, thus forming miniature heads (pl. 9, f. 9).

#### PRIMARY CONIDIA.

The primary conidia are elliptical in shape, and are borne terminally on branched hyphae (pl. 9, f. 10). They measure  $3.8\mu$  by  $1.6\mu$ , average. They are hyaline and colorless. In some cases, where the upper portion of the stalk or head has been broken off new stalks sprout out from the region of the apex in the old one. Branches are also formed without injury.

The fungus penetrates deeply into red gum boards, giving them a dirty appearance, rendering them unfit for making the best grade of boxes, crates, etc. It permeates the medullary rays and many of the larger and some of the smaller vessels of the wood. The mycelium in the wood is generally of a color varying from gray to brown or dark green.

*Graphium rigidum* (Pers.) Sacc.

A species of *Graphium* was found discoloring the sap-

wood of lumber made from *Quercus rubra* L. in Indiana. This, when isolated in pure cultures, grew readily on the sapwood of pine, oak, gum and ash, and on vegetable agar media, also on potato, bean, and other vegetables in test tubes. The fungus corresponds quite closely to the description of *Graphium rigidum* (Pers.) Sacc., with the exception that it has in addition a conidial stage, the conidial clusters taking the closed form. The following emended description of this species is now given from artificial cultures on wood and agar media: —

#### MYCELIUM.

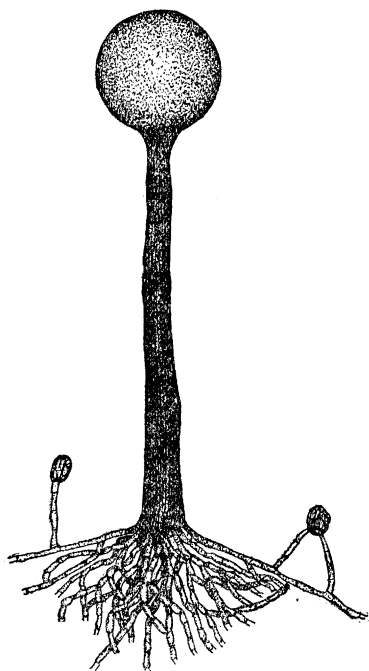
Cultures of both primary and secondary conidia sown in ordinary vegetable agar media germinate in a few hours, and in two days begin to bear an abundant crop of secondary conidia in clusters of a type similar to those of *G. smaragdinum*, which collect as rapidly as they develop into rounded, mucilaginous clusters (pl. 7, f. 7). The filaments of the mycelium are hyaline at first, but in a few days form a brown pigment in the older portion, and throw up stalked heads bearing the primary conidia. These vary from a stalk consisting of two or three filaments with a small globular or flattened oval head, to one consisting of many, with a much larger head (pl. 7, f. 6).

#### SECONDARY CONIDIA.

Secondary conidia are borne on both simple and branching hyphae by abstriction of the ends, and as fast as they are formed adhere together in masses resembling *Cephalosporium*. They are colorless, thin-walled, and are usually free from guttules and granules. They measure  $3\mu$  to  $4.5\mu$  by  $1\mu$  to  $1.5\mu$  (pl. 7, f. 8).

## PRIMARY CONIDIA.

Primary conidia are elliptical, resembling in appearance and form the secondary conidia. They are hyaline, and



*G. RIGIDUM*,  $\times 50$ .  
Stroma, with conidiophores.

measure  $3.5\mu$  by  $1.5\mu$ , average, in size (pl. 7, f. 9, 10). They are borne terminally on alternately branched, hyaline hyphae, and fall together in large, spherical, mucilaginous heads. The heads vary in color from a white to a dingy yellow. They measure, with the mucilaginous drop,  $20\mu$  to  $500\mu$  in diameter. The stalks vary from gray to brown or black, and measure from 1 mm. to 2 mm. in length, and  $10\mu$  to  $40\mu$  in diameter. They are usually solitary at first, but later may become gregarious, or even branched, especially in older cultures where a secondary growth has taken place.

In the study of this species several instances were noted where hyphae bearing the secondary conidia branched directly from filaments connected with the heads bearing the primary conidia, thus giving additional proof of the relation between the two forms of fruiting. (See figure).

2. Species with secondary conidia borne either in simple clusters or in clusters of short branched chains.

*Graphium aureum* n. sp.

The first species of *Graphium* differing in conidial forms

from the previous species was found on discolored boards made from the white pine, *Pinus Strobus L.* This stained the wood a light brown color, less intense than that made by any other species of *Graphium* investigated, with the exception of *G. eumorphum*. The constant presence of short chains in the formation of the secondary conidia was a character that needed the most careful verification, since it was possible that an admixture of *Ceratostomella* with the *Graphium* had taken place. This form of conidia was accepted as a fact only after careful study for six months, with repeated dilution cultures of both primary and secondary conidia in numerous agar poured plates and test tube cultures.

Another difficulty lay in the resemblance of the fungus to species of *Stilbum*, the stroma in its earliest stages being identical with species of this genus, owing to the absence of color. The same difficulty is to be found with others of the species studied. This indicates that the separation of *Graphium* from *Stilbum* on a color basis is not a good one, and that there is no good dividing line between the two genera. Should it be found that species of *Stilbum* have secondary conidia similar to those of *Graphium*, a better basis for generic separation would be found in the types of such conidia.

The following are the cultural characters of this species which is now named *Graphium aureum*:—

#### MYCELIUM.

In colonies grown on agar plates either from primary or secondary conidia there appear in two or three days secondary conidia borne singly or in branched chains from simple, erect conidiophores. These conidia are like those of *Sporotrichum* and of *Ceratostomella*. The mycelium is hyaline at first, changing to yellow, then to light brown. The filaments measure from  $2\mu$  to  $3\mu$  in diameter.

## SECONDARY CONIDIA.

The secondary conidia are borne at first in loose, open clusters which, after standing for a few days in moist air, fall together in rounded masses, adherent to the terminus of the conidiophores (pl. 9, f. 6). They are hyaline, obovate to club-shaped, and are not guttulate except when old. They measure  $4\mu$  to  $8\mu$  by  $1\mu$  to  $2\mu$ , averaging  $5\mu$  by  $1.8\mu$ .

## PRIMARY CONIDIA.

The stalks or stromata bearing the heads of primary conidia are white at first, changing to a yellow color, and often to a dark brown at the base (pl. 9, f. 5). They measure  $50\mu$  to  $750\mu$  by  $10\mu$  to  $90\mu$ , and are composed of parallel filaments measuring  $1\mu$  to  $2\mu$  in diameter. These bear conidia terminally which are held together by mucilage in a flattened, oval head, which is white at first, then creamy yellow, finally a light brown when old and dry. The primary conidia are hyaline, obovate, and measure  $4\mu$  to  $5\mu$  by  $1\mu$  to  $2\mu$  (pl. 9, f. 7).

In cultures on rice and potato tubes tall *Anthina*-like, sterile, branching stalks, or stromatal outgrowths of a light brown color from 1 mm. to 4 mm. in height, are formed. These are very plainly abortive fruiting organs.

*Graphium album* (Corda) Sacc.

A species of *Graphium* was found growing on the wood of the beech, *Fagus atropunicea* (Marsh.) Sudworth. This discolored the wood a brown color. It was found to be a species resembling *G. aureum*, but sufficiently distinct to be separated from the latter species. A culture was sent to Mrs. Flora Patterson, Mycologist of the Bureau of Plant Industry, Washington, D. C., who identified the fungus as probably *Graphium album* (Corda) Sacc., a species which

is imperfectly described. The fungus is now assigned to this species with the following emended description taken from cultural characters: —

#### MYCELIUM.

The growth of colonies from both primary and secondary conidia is much like that of *G. aureum*. The colonies remain hyaline for some time; even the stalked heads show an absence of color for several days after their appearance. Finally the mycelium assumes a gray color, often with a green tinge, and at the same time the stromata assume a darker color. The filaments measure  $2\mu$  to  $3\mu$  in diameter.

#### SECONDARY CONIDIA.

The secondary conidia are of the type of branching chains, resembling *Ceratostomella* (pl. 9, f. 2, 3). They are obovate to cylindrical, hyaline, and measure  $4\mu$  to  $6\mu$  by  $1\mu$  to  $2\mu$ . The conidial clusters are open at first, but in moist air fall together after a few days, forming rounded masses on the ends of the hyphae.

#### PRIMARY CONIDIA.

The stalks bearing the heads are hyaline at first, changing to a gray, finally to brown or black at the base. They are often much swollen at the base, and measure from .3 mm. to 2 mm. in length by  $30\mu$  to  $300\mu$  in diameter. The heads are large and showy, often much flattened, or even recurved at the edges, and measure  $20\mu$  to  $600\mu$  in width (pl. 9, f. 1). Their color is white at first, changing later to a creamy yellow color. The primary conidia are borne continuously on branched hyphae from the ends of the filaments in the head, falling off rapidly as they are formed, into the mucous mass which surrounds the head. They are hyaline, and measure  $3\mu$  to  $5\mu$  by  $1\mu$  to  $1.5\mu$  (pl. 9, f. 4).



## 2. HORMODENDRON.

An intense black color in irregular blotches often occurs on new pine, elm, ash, gum, oak, and other kinds of lumber, when sawn under a moist condition, during warm weather. A large number of such spots were examined, and the fungi present isolated for investigation. Species of *Hormodendron*, *Hormiscium* and *Alternaria* were found present. Two species of *Hormodendron* were identified, viz: *H. cladosporioides* (Fres.) Sacc. and a species not agreeing with any description previously published, and which is here given the name *H. griseum*.

Inoculations of these two species were made upon pine, elm, oak, ash and gum wood. Both species were found to produce superficial black stains, but neither penetrates deeply into hard woods. On the other hand, either species penetrates deeply boards made from the red gum tree, *Liquidambar styraciflua* L. Under the microscope the conidial clusters of the two species closely resemble each other, but in cultures the gross appearance of the colonies is constantly quite different, the former varying from a yellow-green to a dark olive, or almost black, the latter from a light gray to a color approaching black; other differences were noted in the length of the conidiophores and of the conidial chains.

*Hormodendron cladosporioides* (Fres.) Sacc.

The species of *Hormodendron* most commonly found on boards is described here from artificial cultures, and assumed to be *H. cladosporioides*. Two other species occurring on wood, which have been previously described, but at a later date, are *H. atrum* Bon. and *H. elatum* Hartz. From the description of these two latter species it would be very difficult to separate the one from the

other, or either of them from *H. cladosporioides*. The description of our fungus might fit any of the three, but as the last named species is the older, its name will hold valid.

The following cultural characters were obtained from a large number of cultures on agar media, most of which were made from pine-decoction alone, or from the juices of vegetables combined with a pine-decoction:—

#### MYCELIUM.

Conidia sown on agar plates germinate and produce small, gray colonies visible to the eye in 24 hours. In three to four days the colonies begin to send up erect sporophores, bearing clusters of short septate branches, which are often branched again and from the ends of which branched chains of olive-colored conidia are formed (pl. 10, f. 1). The younger portions of the colony assume a yellow-green color, which rapidly changes to an olive, and finally to an almost black color, while the surface has a velvety appearance. The filaments are coarsely granular in the older portion, and are often constricted at the point of septation. They measure 2 to  $8\mu$  in diameter. The sporophores measure  $100\mu$  to  $400\mu$  in length, and  $3\mu$  to  $4\mu$  in diameter; the branches are 1- to 2- rarely 3- septate, and measure  $6\mu$  to  $15\mu$  by  $3\mu$  to  $5\mu$ .

#### CONIDIA.

The conidia are borne in simple chains of 2 to 6, rarely more; they are not granular or guttate except when old, and vary in shape from a blunt to a pointed oval. They measure  $3\mu$  to  $7\mu$  by  $2\mu$  to  $4\mu$ . When mature, both the hyphal branches and the conidia are of a dark olive color.

The work of Planchon\* indicates that *Hormodendron*

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\* Planchon, Louis. Influence de divers milieux chimiques sur quelques champignons du groupe des Dématiées. Annales des Sciences Naturelles. Botanique. 11: 1-256. pl. 1-4. (1900).

*cladosporioides* (Fres.) Sacc. and *Cladosporium herbarum* Link are identical. In some of our cultures forms intermediate between the two, and identical with the latter, were present (pl. 10, f. 1), but in no case were septate conidia noted, such as are given in the description of *C. herbarum*, the septation being confined to the hyphae which bear the conidia. If, however, the modified hyphal branches be mistaken for conidia, it is easy to assume them to be the septate conidia of *Cladosporium*. Time was not had to apply all the culture methods and media of Planchon to our species. Enough of the forms described by him in his study of these two fungi have been found in our cultures to verify his work; but the question still remains as to whether he is correct in assuming the fungus to be a *Cladosporium*. This hinges upon whether the conidia-like branches bearing the conidia may themselves function as conidia; but even this might not settle the question, since it is a well-known fact that in most of our imperfect fungi any portion of the young mycelium when broken up may be able to reproduce the fungus. This being the case, we will for the present use the name *Hormodendron* here to designate our plant.

*Hormodendron griseum* n. sp.

The other species of *Hormodendron* studied was found growing on the wood of *Liquidambar Styraciflua* L., *Pinus echinata* Mill., and *Fraxinus Americana* L. The colonies of this species on both wood and agar media were of greater height, and of a different color when mature, as compared with those of *H. cladosporioides*. The mycelium is often hyaline, or nearly so in many portions, giving the colony a gray appearance, even though the conidial clusters are of a dark olive color. These two species of *Hormodendron* were grown carefully for nearly a year in parallel sets of cultures, with no reversion of the one type to the other,

which was looked for. The following description of *H. griseum* is made from cultures grown on the same media from which the characters of *H. cladosporioides* previously given were obtained:—

#### MYCELIUM.

The conidia of this species germinate readily, and similarly to those of the preceding species, but the colony is a day or two longer in producing conidia than that of the former species. The habit of fruiting is the same, but the conidia are borne in longer chains. The mycelium is coarsely granular, and thick-walled in the older filaments, which measure from  $3\mu$  to  $10\mu$  in diameter. The sporophores or fruiting hyphae measure  $200\mu$  to  $800\mu$  in length, and  $3\mu$  to  $4\mu$  in diameter. The hyphal branches are in two series, and are from 1- to 3- septate, measuring  $6\mu$  to  $14\mu$  by  $3\mu$  to  $4\mu$ .

#### CONIDIA.

The conidia are borne in branching chains of 2 to 10, and are formed from the ends of the hyphae by abstriction (pl. 10, f. 2). They are of a sooty color, pointed oval, and measure  $3\mu$  to  $6\mu$  by  $2\mu$  to  $4\mu$ .

### 3. HORMISCIMUM.

#### *Hormiscium gelatinosum* n. sp.

In the study of the black stain of pine, elm and red gum boards at least one species of *Hormiscium* was found causing a very intense black stain, especially in sapwood. It is with some hesitancy that this fungus is named *Hormiscium* in view of the great resemblance of the mycelium on agar plate cultures to some of the forms of *Dematium pululans* de Bary, found by Planchon\* in his study of the

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\* Planchon, Louis. *l. c.*

latter fungus, and related fungi. The finding of chains, or round conidia borne in chains, on short hyphae (pl. 11, f. 7), and the identification of the fungus as *Hormiscium*, by Mrs. Flora Patterson, has led to the placing of the species under the genus *Hormiscium*. This form differs from other species of *Hormiscium* in its gelatinous nature and dimorphous conidia, and is now called *Hormiscium gelatinosum*, with the following cultural characters: —

The fungus grows readily on many kinds of media, especially upon agar media from vegetable decoctions, upon various substances containing starch, and upon the sapwood of pine, oak, gum, and other trees. It makes only a scanty growth on the heartwood. On potato and rice tubes it covers the surface with a black, shiny growth of slimy, matted filaments.

#### MYCELIUM.

When conidia of either form are sown on agar plates the colonies which result resemble very much those of some yeasts and bacteria. The growth of the colony is slower than that of most fungi. The form of the colony is variable. Where the conditions are not favorable the colony is rounded, cream-colored, mucous, growing with a roughened surface and a wavy edge. In more favorable conditions, on better media, etc., the edge of the colony becomes fimbriate or toruloid (pl. 11, f. 4). The shape of the colony thus apparently depends directly upon the conditions under which it grows. The filaments of the mycelium are fewer in relative proportion to the bulk of the colony in the younger forms than in the older forms. In older colonies, near the edge, filaments predominate. They are toruloid in form, the cells are very irregular in shape, varying from long, cylindrical, almost hyaline cells (pl. 11, f. 7), to beaded forms (pl. 11, f. 8) of a dark olive color. The former vary in diameter from  $2\mu$  to  $8\mu$ ; the latter,

from  $5\mu$  to  $10\mu$ . The latter form are of the nature of chlamydospores, having thick walls, and from one to two cells. These sometimes germinate, and apparently may function as resting spores.

#### CONIDIA.

Two forms of conidia are borne on the mycelium, one of these being the hyaline form (pl. 11, f. 5), which buds both from the cells of the mycelium and from other conidia during the earlier growth of the colony. These are deeply imbedded in mucilage, and germinate readily, if not too old, in agar plate cultures. They are ephemeral, losing their power of germination in a few weeks. They are elliptical in shape, and measure  $8\mu$  to  $12\mu$  by  $3\mu$  to  $5\mu$ , and are rarely septate. Among the hyaline conidia, as the colony grows older, brown forms of the same shape soon occur (pl. 11, f. 6), and finally become very abundant. These are thick walled, and do not appear to germinate as readily as the hyaline type. They probably function as resting spores. They are sometimes septate, but more often have one cell. They measure  $10\mu$  to  $14\mu$ , by  $5\mu$  to  $6\mu$ . Another form of conidia of the true type of *Hormiscium* (pl. 11, f. 7) is found borne from the filaments in the older portions of the extremities of the colony, and on the filaments in cultures upon wood. These are globose in form, of a dark olive color, not rugose, and are borne from short hyphae in chains of two to several. They measure from  $7\mu$  to  $12\mu$  in diameter. These chains hold together tenaciously, and do not break apart like those of related genera.

#### 4. OTHER WOOD-BLACKENING FUNGI.

A number of other fungi were noted, which add to the stains which occur superficially on lumber, especially on the sapwood. Several of these were identified, which apparently discolor the wood only by the color present either

in the mycelium or the fruiting bodies. None of them are considered as important in their effects upon the value of the lumber, as the mycelium does not penetrate deeply into the wood. Those identified were: *Alternaria tenuis* Nees, *Stachybotrys alternans* Bon., *Chaetomium* sp., *Aspergillus niger*, *Stemonites* sp., and *Gliocladium* sp.

### III. WOOD-REDDENING FUNGI.

#### 1. *Penicillium*.

The fact that *Penicillium* might stain wood was brought to our attention during the year 1903. Some cultures of *Ceratostomella exigua* were contaminated with a lemon-colored fungus, which, under certain conditions, stained the wood an orange red color. Upon isolation it was found to be a *Penicillium*, which was dimorphous, having a fertile mycelium with gray-green colored fruiting clusters, and a sterile mycelium which assumed either a lemon color or an orange red color, varying with the reaction of the medium upon which it was grown, the former color being present on acid media, and the latter on alkaline.

This led to the study of *Penicillium* from various sources, and from different kinds of wood. Some were found to stain pine sapwood, under certain conditions, an orange red color, others a crimson red, and still others, a color intermediate between the two. At least three species of *Penicillium* were found which were proven to stain wood. These were often intermingled, sometimes with each other, and at some times with *Fusarium*. This complicated the problem, and the difficulty was further increased by the fact that it is hardly possible to name correctly the color-producing species of *Penicillium* without a long series of cultures. The investigations of Dierck \*

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\* Dierck, F. Essai de révision du genre *Penicillium*. Ann. de la Soc. de Brux. 1901.

and that of Thom \* have shown that even when the form characters of two forms of *Penicillium* are alike, there may be distinct differences in the color of the fungus on certain different kinds of media. The former has made a revision of the genus *Penicillium*, based chiefly on physiological characters, but also taking into consideration the strictly morphological. The latter in studying the species of *Penicillium* which were found concerned in the ripening of the various kinds of cheese found great difficulty in naming them, owing to the fact that what may be morphologically the same species may not have the same effect on the chemical properties of freshly made cheese, owing to varying action of different strains or races of *Penicillium* on the organic compounds present.

*Penicillium aureum* Corda.

Of the two species of *Penicillium* studied, the most common was identified finally as *Penicillium aureum* Corda. It is dimorphous, as has already been mentioned, and is one of the common species found on pine wood. It was also found in cultures from gum, oak, and other woods. A culture sent to Mrs. Flora Patterson was identified as *P. aureum*, thus verifying our observation.

This species grows readily on the fresh sapwood of our most common forest trees, upon bouillon, and various vegetable decoction agar media, upon potato, starch, and other similar substances. The following cultural characters are taken from both natural and artificial cultures.

MYCELIUM.

Cultures upon agar media develop rapidly from conidia, the mycelium usually becoming visible within a day as a

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\* Thom, C. Some suggestions from the study of dairy fungi. Jour. Myc. 11 : 117-124. (1905).



small, white patch on the surface. In two or three days, sporophores are thrown up from the prostrate mycelium. These bear branching hyphae which form terminally by abstriction, simple, continuous chains of conidia. This portion of the colony is usually gray to blue green in color, varying with the medium. The filaments of the mycelium in rich agar media often bear swollen cells, resembling chlamydospores in shape (pl. 11, f. 2), but which have not been observed to form the thicker wall, characteristic of such spores. Under the same condition, the mycelium becomes fluffy, and many filaments unite together in strands, forming *Coremium* clusters. The filaments ordinarily measure  $3\mu$  to  $4\mu$  in diameter, and contain elongated cells. On the other hand, the swollen cells are spherical, with a diameter of  $4\mu$  to  $8\mu$ .

Upon older cultures, especially upon wood, there are formed rounded tufts or clumps of curled and distorted hyphae and filaments, which are either lemon-yellow or orange-red in color, varying with the acidity or alkalinity of the culture medium. The color is due to the secretion of a pigment in the form of granules on the surface of the filament, evidently by exudation (pl. 11, f. 3). This pigment is readily soluble in slightly acid or alkaline water, in hot alcohol, and in some other solvents. It is red when alkaline and yellow when acid. The fungus, as a rule, when grown artificially, overcomes a moderate amount of alkalinity by the formation of an acid, and as a result, most cultures assume the lemon-yellow color when old. In cultures on wood, especially sapwood, the pigment is carried into the wood cavities by absorption, thus staining the wood. The color, although bright at first, fades as the culture grows old and dries out.

#### CONIDIA.

The conidia are borne on conidiophores which vary in length from  $100\mu$  to  $500\mu$ , with a diameter of  $3\mu$ , or slight-

ly more. These bear terminally a whorl of branched hyphae. The hyphal branches of either the first or second series average  $12\mu$  by  $2\mu$ . The conidial cluster is loose, and spreads out widely, averaging  $100\mu$  to  $350\mu$  by  $35\mu$  to  $70\mu$  (pl. 11, f. 1). The conidia are pointed oval in shape, varying from  $3\mu$  to  $4\mu$  in length, by  $1.5\mu$  to  $2\mu$  in diameter. They are borne in simple chains of 40 to 80. They have a blue-green color, and contain a pigment soluble in hot alcohol, which does not change its blue-green color either by the addition of an acid or alkali.

*Penicillium roseum* LINK.

A species of *Penicillium* which resembles *Penicillium roseum* Link was first found mingled with *P. aureum*. This fungus secretes a crimson red pigment which discolors sapwood. The work of investigation is not completed, and it is thought best not to give further details concerning this fungus at present. A third species has not been identified.

2. FUSARIUM.

The species of *Fusarium*, identified as *Fusarium roseum* Link, was isolated from pink, red and violet blotches found on new pine lumber taken from lumber piles near Ashland, Wis., in 1905. This grew readily under artificial culture, and upon inoculation was found to be the cause of the blotches upon the lumber. The boards from which the original cultures were taken were sawn from trees of *Pinus Strobus* L., and *P. resinosa* Ait. Similar stains have been found on pine boards from the southern United States made from *Pinus palustris* Mill., and *Pinus echinata* Mill., also on pieces of wood from *Pinus Virginiana* Mill.

This fungus, like other species of *Fusarium* that have been studied, varies in form, color, and habit of growth, depending upon the substratum upon which it grows. The

fruiting bodies are usually borne at or above the surface of the medium upon which it is cultivated. The following is a résumé of the cultural characters of the fungus:—

#### MYCELIUM.

The spores of the fungus germinate in a very short time, usually a few hours, and in thirty-six hours small white colonies are visible upon the surface of agar media. The first mycelium that forms is rather sparse, but thickens as the colony grows older. Two types of conidia are borne on the fertile hyphae, which are formed in a short time after germination of the spores. These are the microconidia (pl. 12, f. 1, 2, 3), which are found very numerous in all parts of the mycelium, except in the newest growth, and the macroconidia (pl. 12, f. 4, 5), which are found sparsely only in the older portions of the mycelium. Chlamydospores are formed abundantly in all the older portions of the colony about the time the growth of the mycelium is checked by a limitation of nutrition, being formed usually with or a little later than the macroconidia. The anastomosing of filaments is quite frequent (pl. 12, f. 8).

#### MICROCONIDIA.

Microconidia are formed in great numbers on various vegetable media, such as agar media made with plant decoctions. They are usually borne on hyphae which branch alternately from the mycelium. They are formed by the abstriction of the ends of hyphae, and where they are not disturbed by air currents or some sudden shock, they adhere to each other side by side in clusters which assume a form of fruiting resembling *Cephalosporium* (pl. 12, f. 3). These microconidia are at first one-celled. A septum is often formed before the spore becomes detached from the hypha, and later many of the one-celled conidia that have fallen off form septa. The two-celled form

slightly resembles the conidia of *Cephalothecium* (pl. 12, f. 3).

The microconidia are oval to elliptical in shape, varying in length from  $8\mu$  to  $14.5\mu$ , and in diameter from  $3\mu$  to  $6\mu$ . They are hyaline, colorless, thin walled, and are usually uninucleate. Many are slightly curved.

#### MACROCONIDIA.

The macroconidia are of the *Fusarium* type, and vary considerably in shape and size. They are usually formed by abstriction of the ends of short, swollen, branching hyphae, and are from two- to four-celled, straight or curved, with tapering, rather blunt ends, varying in length from  $19\mu$  to  $30\mu$ , and in diameter from  $3.5\mu$  to  $6\mu$ . Both microconidia and macroconidia germinate readily on all ordinary agar culture media, the time varying with the medium. The macroconidia are rarely found attached to the hyphae when fully mature.

#### CHLAMYDOSPORES.

Both terminal and intercalary chlamydospores are formed singly or several in series (pl. 12, f. 6, 8, 9). They are formed by the enlargement of the cells of the mycelial filaments. Their development is more gradual than that of the conidia, and the cell wall is much thicker. In shape they are spherical, or slightly oval. The cell contents are granular, and vary in color from yellow to dark brown. The diameter of the spores averages  $12\mu$ . When the macroconidia start to germinate, and are hindered by a sudden drying out, or other adverse conditions, it is not unusual for one of the cells to form a chlamydospore (pl. 12, f. 5); these are rarely mature, and have never been observed to germinate.

#### SCLEROTIA.

Sclerotia are produced in cultures on boiled potato and

rice. They vary in color from dark green to dark brown. As they grow older they become rather hard and dense, but bear no definite fruiting bodies.

#### PERITHECIA.

In all of our cultures no perfect form of fruiting has been found, although the fungus has been grown on all the common culture media. If such form exists, our failure to find it is due to the fact that natural conditions were not imitated closely enough to produce it.

#### THE FORMATION OF COLOR.

On pine sapwood, a red or purple color is produced, which in old cultures fades to a dirty brown. A number of species of *Fusarium* are now being grown on pine wood for comparison with this, and the results will be published at a later date. *Fusarium roseum* secretes a soluble pigment which is taken up by the adjacent wood cells, staining them lightly with a red or purple color, which varies with the acidity or alkalinity of the wood. In natural wood, which is slightly acid, the color varies from a pink to a lilac. In cultures where there is a profuse growth of the mycelium the colored chlamydospores and mycelium modify the appearance of the wood, giving it a dull color in contrast with the original brighter one.

#### CAUSES OF COLOR IN WOOD STAINED BY THE FUNGI INVESTIGATED.

##### 1. CERATOSTOMELLA.

The cause of the blue-gray or blue-black stain in wood penetrated by the mycelium of *Ceratostomella* lies in the color of the filaments of the fungus, which exudes no stain from its mycelium. The dark brown pigment in the walls

of the filaments is insoluble in alcohol, ether, chloroform, benzol, alkalis and acids. The brown color of the fungus apparently contains traces of a blue pigment whose color is transmitted by the wood cells of pine more readily than the brown color.

## 2. GRAPHIUM.

The mycelium of *Graphium* imparts a dingy gray or brown, or even a black color to the wood it enters. This is caused by the color of the mycelium, and is due to no soluble pigment. The cells of the wood remain unstained.

## 3. HORMODENDRON AND HORMISCIMUM.

The cells of wood penetrated by these wood-blackeners are not stained, the color being due to the presence of the mycelium of the fungi. It is not yet known whether the color is one that may be extracted.

## 4. PENICILLIUM.

The species of *Penicillium* discoloring wood form a soluble red or yellow pigment which is taken up by the cell walls of the wood, giving them a red or yellow stain which fades when the wood dries out, but is increased in intensity when it is moistened again.

## 5. FUSARIUM.

The wood stained by *Fusarium* is discolored both by a soluble pigment which is secreted by the fungus and taken up by the wood cells and by the presence of colored hyphae and chlamydospores.

The investigation of color production by these fungi will be carried on in the future, and it is hoped that the nature and composition of some of these pigments may be discovered, and that other facts may be found worthy of publication.

## KEY TO THE SPECIES OF FUNGI DESCRIBED IN THIS PAPER.

A. Colonies at first white, changing color later. First fruiting stages, clusters of hyaline conidia borne on upright hyphae which usually collect together in rounded masses under moist conditions.

a. Staining wood bluish, black or brown; with beaked perithecia.

CERATOSTOMELLA.

1. Conidia borne in short, branching moniliform chains on upright hyphae.

\* Beaked ostiolum more than twice the height of the perithecium.

† Fringe to ostiolum terminal.

§ Terminal filaments short and often thickened.

Perithecium smooth or sparsely hirsute. *C. pilifera*.

Perithecium often with outgrowths. *C. Schrenkiana*.

Perithecium with glandular hairs. *C. echinella*.

§§ Terminal filaments long and slender.

*C. capillifera*.

‡ Fringe to ostiolum often supplemented by additional rings beneath. *C. pluriannulata*.

\*\* Beaked ostiolum only once or twice the height of the perithecium.

Terminal filaments short and thickened. *C. minor*.

Terminal filaments lengthened and slender. *C. exigua*.

2. Conidia borne continuously, either singly or in moniliform chains.

Perithecium with conical spines. *C. moniliformis*.

b. Staining wood gray to brown or black; mycelium bearing stromata or stalked heads. GRAPHIUM.

1. Secondary conidia resembling those of *Sporotrichum*.

Stroma finally brown to black, heads white to brown.

*G. ambrosiigerum*.

Stroma finally brown or olive, heads greenish. *G. eumorphum*.

Stroma finally dark green to black, heads gray. *G. atrovirens*.

2. Secondary conidia differing from those of *Sporotrichum*.

\* Conidia formed continuously, falling together as rapidly as formed.

Stroma finally dark green to black, heads white to gray or green. *G. smaragdinum*.

Stroma finally gray to black, heads white to creamy yellow.

*G. rigidum*.

\*\* Conidia in simple whorls or in whorls of branching moniliform chains.

Stroma finally yellow to brown, heads yellow to light brown. *G. aureum*.

Stroma finally brown to olive, heads white to yellow.

*G. album*.

B. Colonies at first gray, later brown or black, conidia dark colored, borne in branched chains in clusters from upright hyphae.

HORMODENDRON.

Velvety colonies, yellow-green at first, then olive to black.

*H. cladosporioides*.

Furry colonies, light to dark gray, finally almost black. *H. griseum*.

C. Colonies yeast-like at first, mucilaginous, finally turning brown or black.

HORMISCIMUM.

Mycelium cream-colored, then brown, conidia dimorphous, chlamydo spores present.

*H. gelatinosum*.

D. Colonies gray, green, yellow or red, conidia hyaline or slightly colored, borne in clusters of simple chains from branched upright hyphae.

PENICILLIUM.

Mycelium dimorphous, lemon-yellow to orange in one form, gray or blue-green in the other; staining wood yellow or red. *P. aureum*.

Mycelium white or pink to red; staining wood crimson.

*P. roseum* (?)

E. Colonies white, often changing to red or lilac, microconidia one- to two-celled, macroconidia several celled, fusiform or sickle-shaped.

FUSARIUM.

Staining wood pink, red, or purple, or even brown in old cultures.

*F. roseum*.

#### EXPLANATION OF PLATES.

The illustrations are from camera lucida drawings by the author except for pl. 6, f. 7, 8, drawn by Miss Laura L. Eames. Throughout, perithecia and their tips, and stromata, are enlarged 50 diameters; and mycelial structures, conidiophores, conidia, and ascospores are enlarged 500 diameters.

Plate 3. — Ostiola of *Ceratostomella*. 1. *C. capillifera*. 2. *C. exigua*. 3. *C. echinella*. 4. *C. minor*. 5. *C. moniliformis*. 6. *C. Schrenkiana*. 7. *C. pluriannulata*. 8. *C. pilifera*.

Plate 4. — *Ceratostomella Schrenkiana*. 1, 2. Conidial clusters. 3. Ascospores. 4. Young perithecium, with conidiophores from some of the older hyphae. — *C. pilifera*. 5, 6. Conidial clusters. 7. Germinating conidia. — *Cephalosporium*-like aggregations are shown in figures 1, 5, and 6.

Plate 5. — *Ceratostomella pluriannulata*. 1. A conidial cluster. 2. An ascus and ascospores. — *C. moniliformis*. 3. Conidial clusters. 4. A moniliform chain of conidia formed like arthrospores, two conidia germinating. 5. A *Cephalosporium*-like aggregation of conidia termi-



nating two moniliform chains. — *C. minor*. 6. Perithecium almost mature. 7. An ascus and ascospores.

Plate 6. — *Ceratostomella echinella*. 1. A conidial cluster. — *C. capillifera*. 2. A conidial cluster. 3. *Cephalosporium*-like aggregations of conidia. — *C. exigua*. 4, 5. Conidial clusters, one having become massed. 6. Conidia. 7. Ascospores.

Plate 7. — *Graphium eumorphum*. 1. Stroma with heads. 2. Clusters of secondary conidia. 3. Secondary conidia. 4. Parallel hyphae composing a portion of a stromatic stalk. 5. Branches of hyphae bearing primary conidia from head. — *Graphium rigidum*. 6. Stroma with head. 7. Conidial masses on surface of an agar culture, secondary conidia. 9. Primary conidia. 10. Germination of primary conidia.

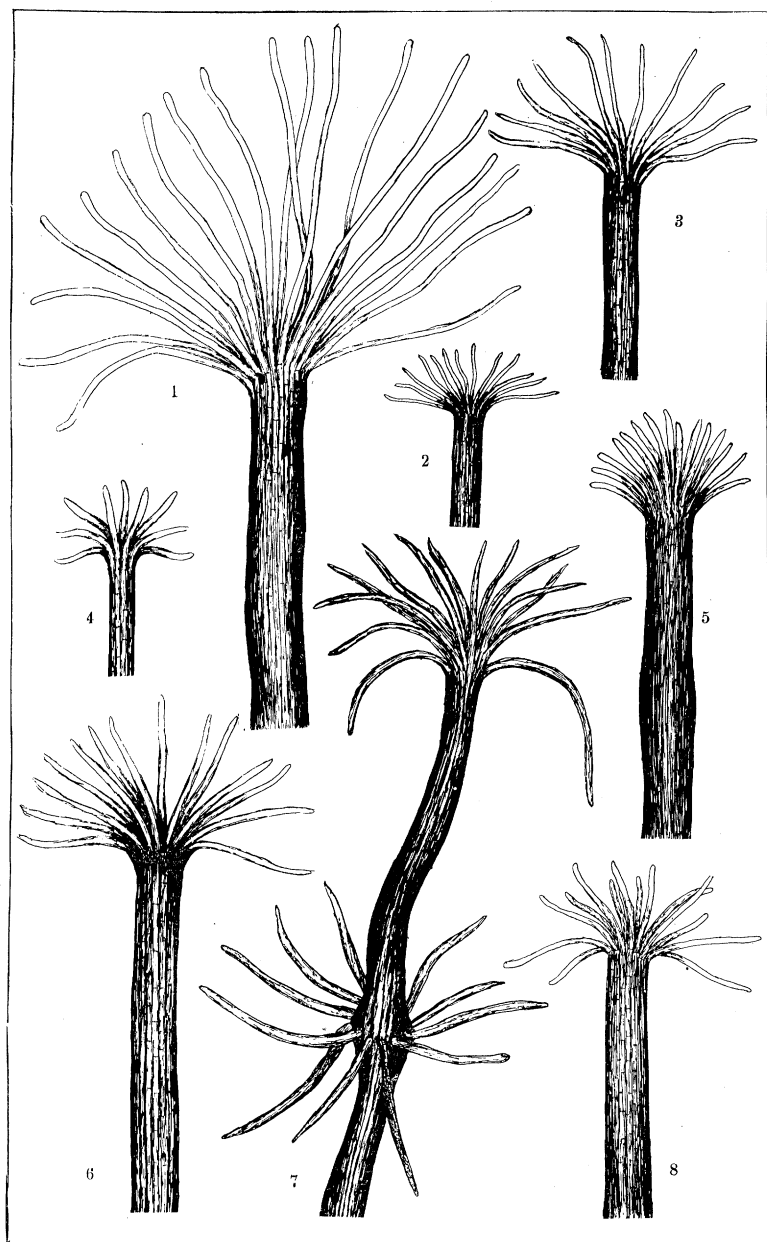
Plate 8. — *Graphium atrovirens*. 1. Stromata with heads. 2. Clusters of secondary conidia. 3. Compound cluster, or side clusters of secondary conidia. — *Graphium ambrosiigerum*. 4. Stroma with heads. 5. A cluster of secondary conidia. 6. Stroma, origin of stalk bearing head. 7. Filament bearing primary conidia from head.

Plate 9. — *Graphium album*. 1. Stromata with heads. 2. Clusters of secondary conidia. 3. Compound cluster of secondary conidia. 4. Primary conidia. — *Graphium aureum*. 5. Stromata with heads. 6. Conidial clusters and masses of secondary conidia. 7. Primary conidia. — *Graphium smaragdinum*. 8. Stromata with heads. 9. Clusters of secondary conidia. 10. Filaments bearing primary conidia from head.

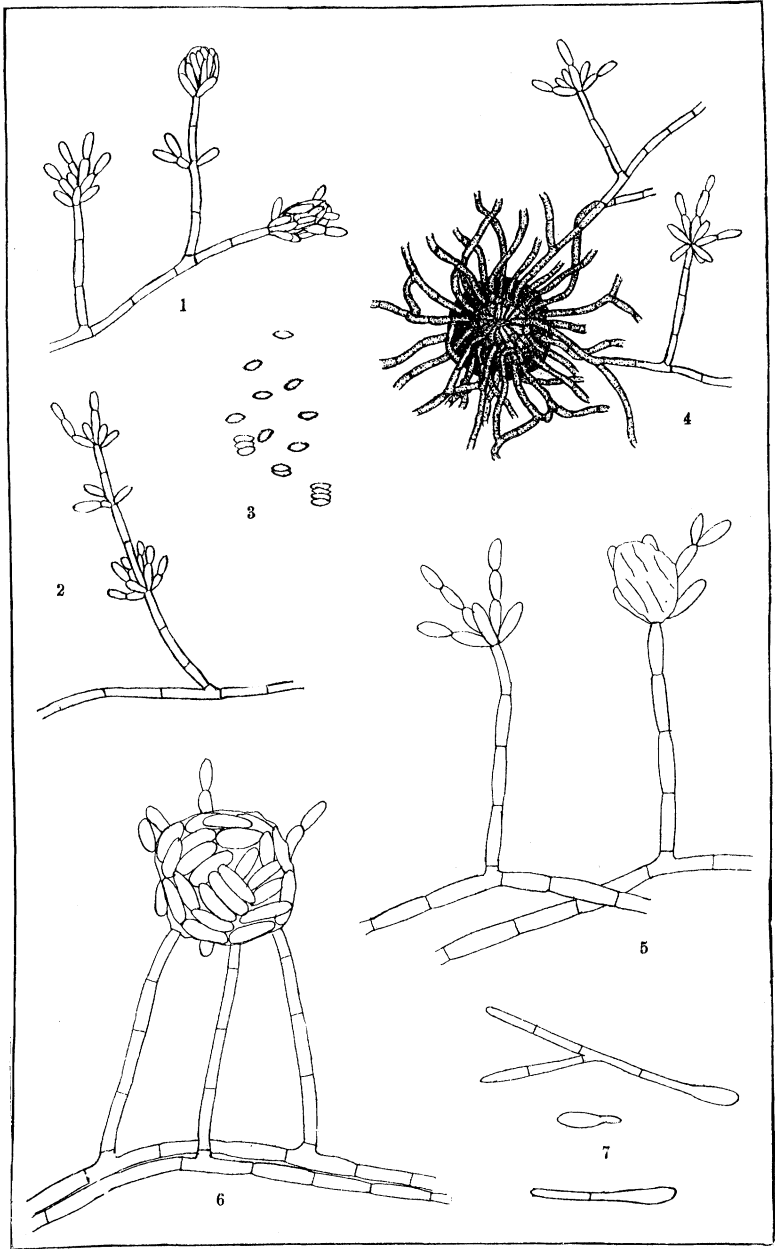
Plate 10. — *Hormodendron cladosporioides*. 1. Conidial clusters. — *H. griseum*. 2. Conidial clusters, the smaller showing a *Cladosporium* type.

Plate 11. — *Penicillium aureum*. 1. A portion of a conidial cluster with two complete chains of conidia, the remainder being incomplete and the conidiophore drawn in two pieces. 2. A portion of the mycelium from a culture of pine-decoction agar showing swollen cells suggestive of chlamydospore formation. 3. A filament rugose with a deposit of a colored granular exudation. — *Hormiscium gelatinosum*. 4. A colony on agar showing the toruloid form, (enlarged 6 times). 5. A hypha with hyaline yeast-like conidia. 6. Brown form of loosely attached conidia. 7, 8. Conidia in chains borne on short hyphae, the *Hormiscium* type.

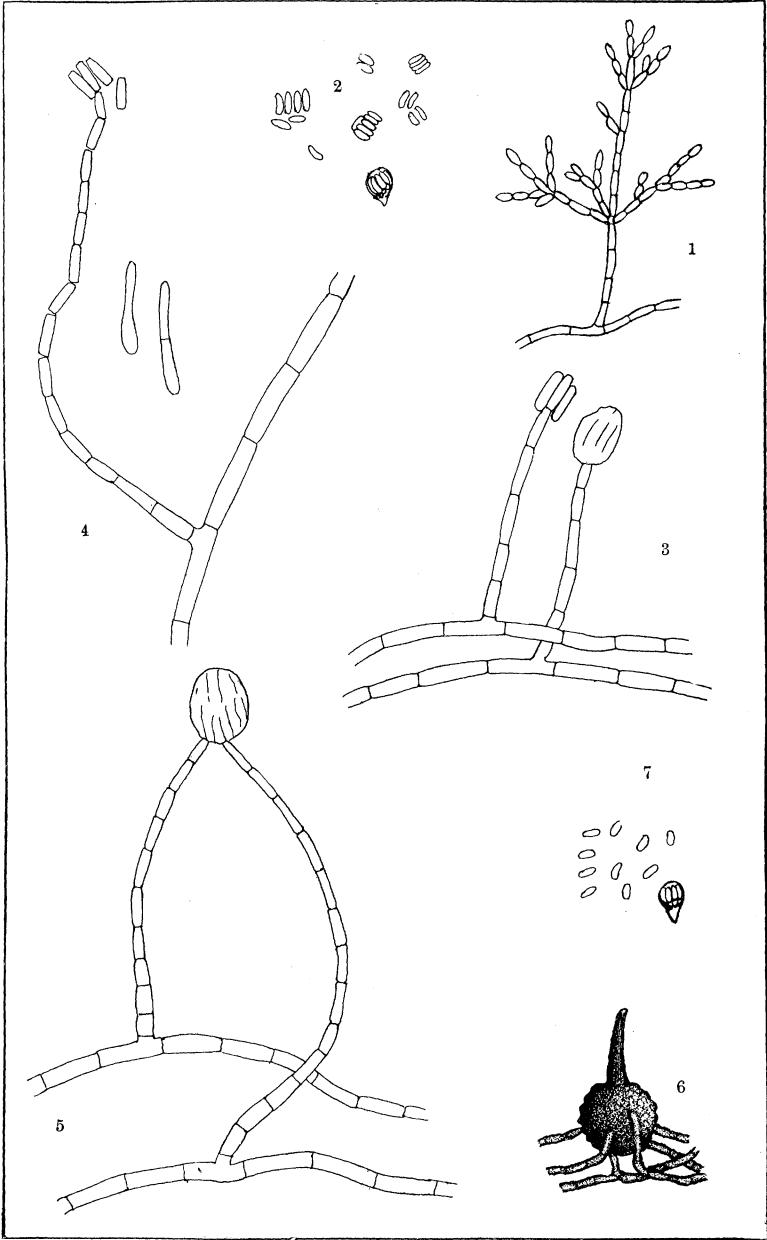
Plate 12. — *Fusarium roseum*. 1, 2. Microconidia, *Verticillium*-like forms. 3. Microconidia aggregated in *Cephalosporium*-like masses and at the same time becoming uniseptate. 4. Microconidia one- to two-celled, macroconidia three- to four-celled. 5. Formation of abortive chlamydospores caused by the arrested germination of macroconidia. 6, 7, 8. Chlamydospore formation.



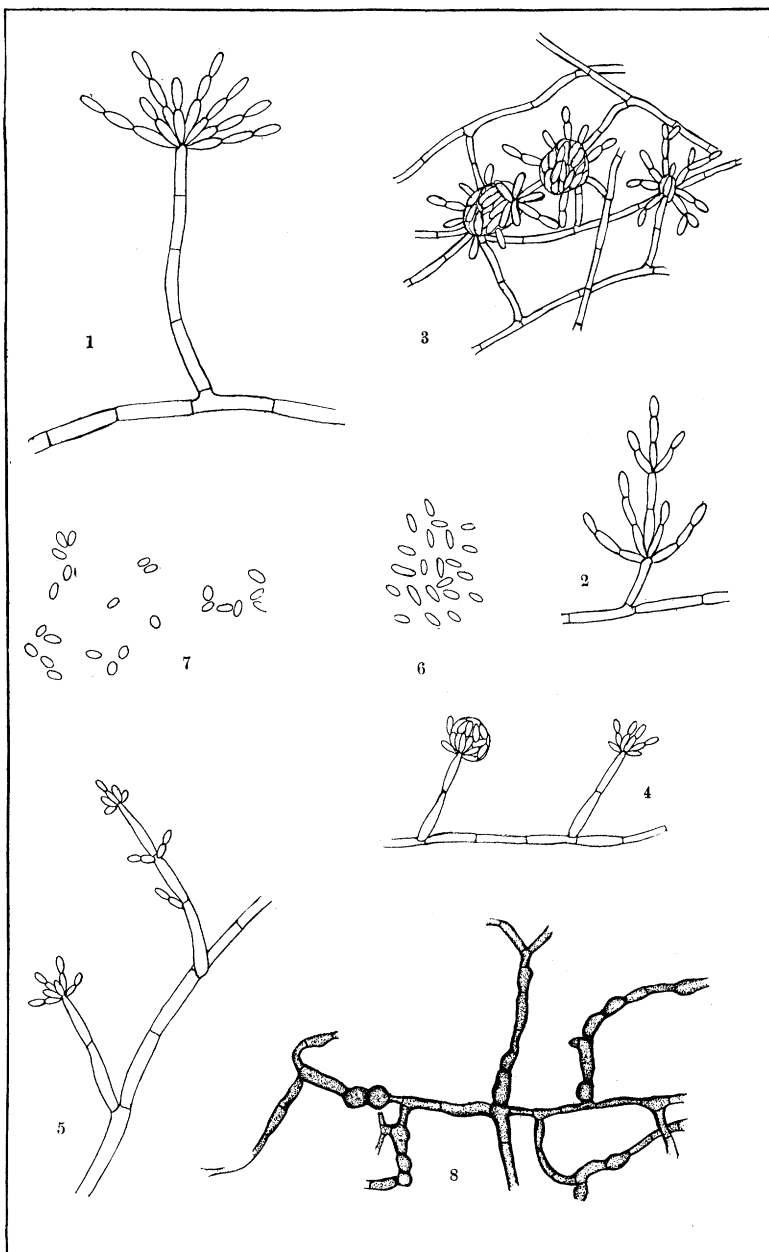
CERATOSTOMELLA.



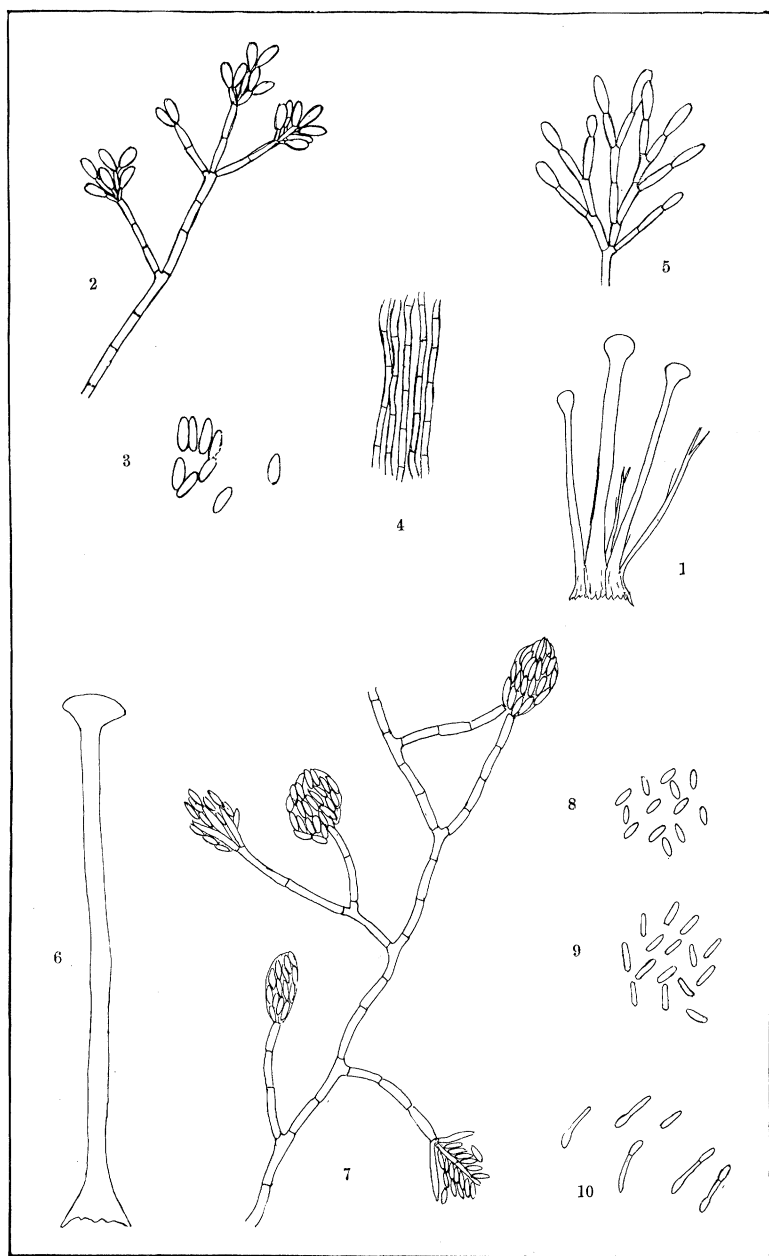
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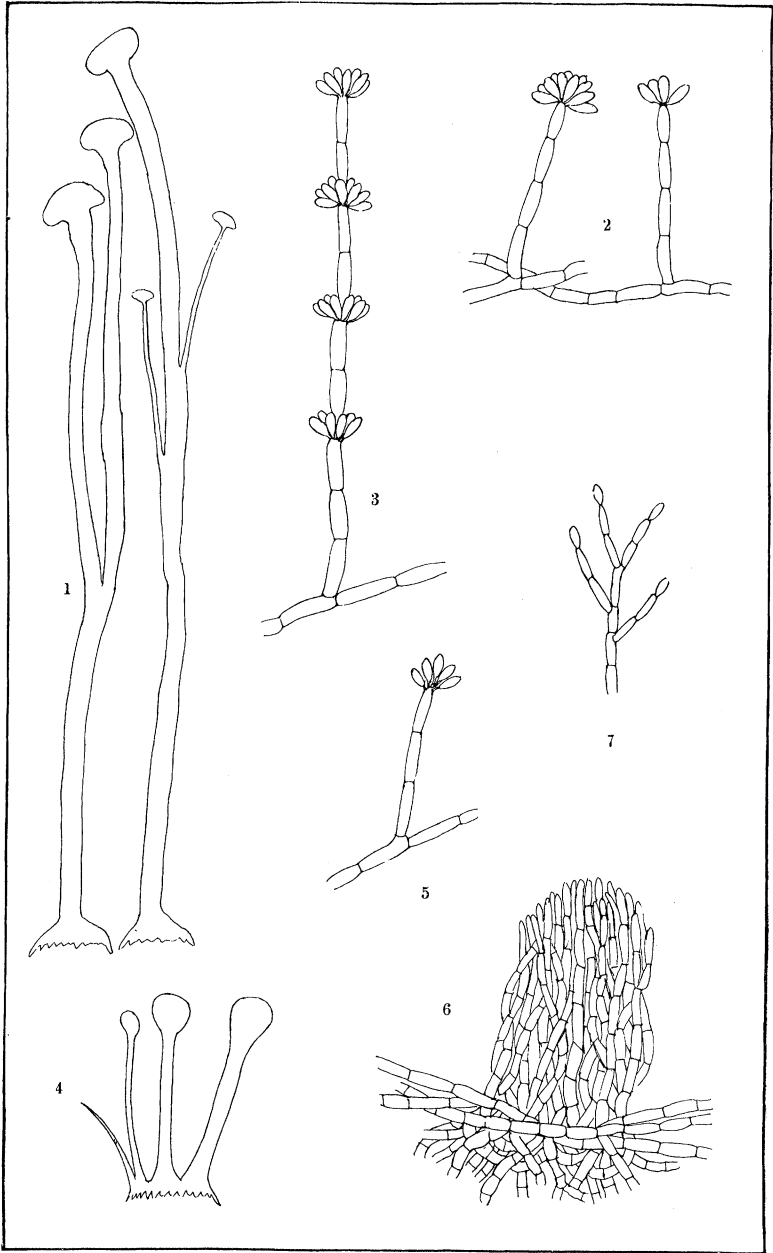
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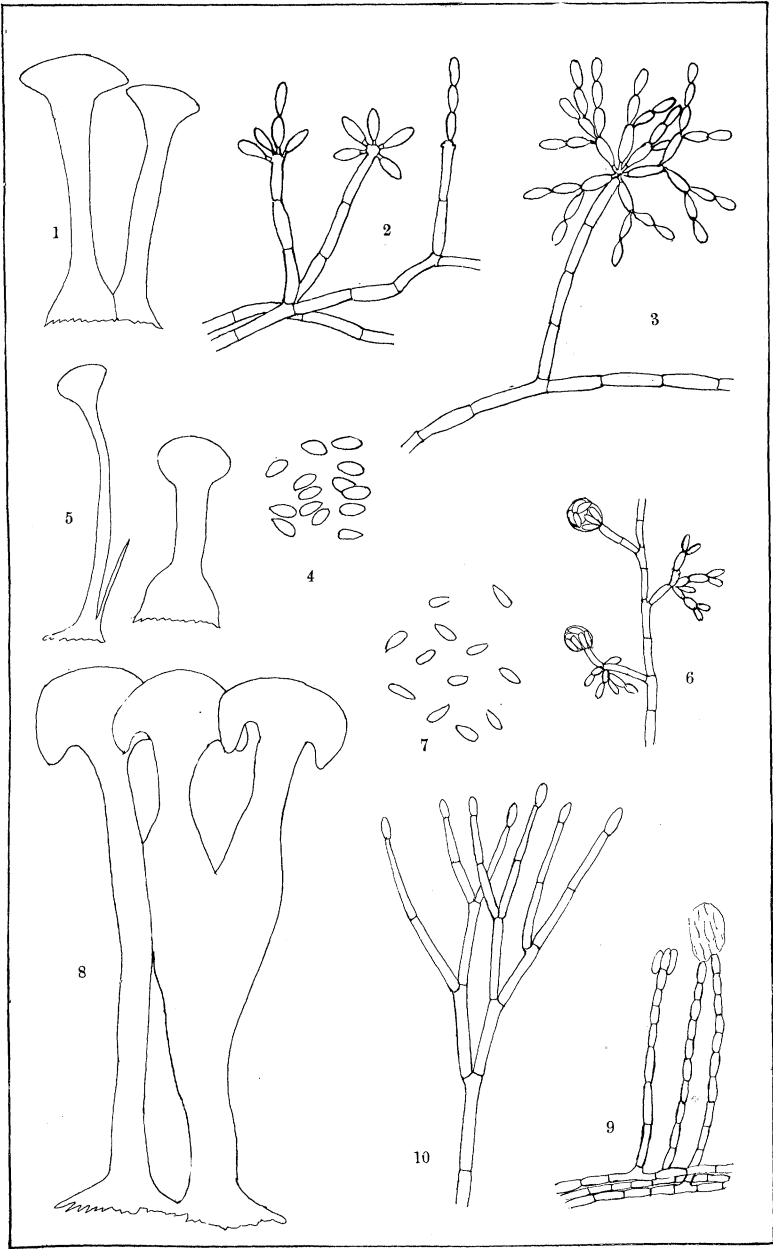
CERATOSTOMELLA.



GRAPHIUM.

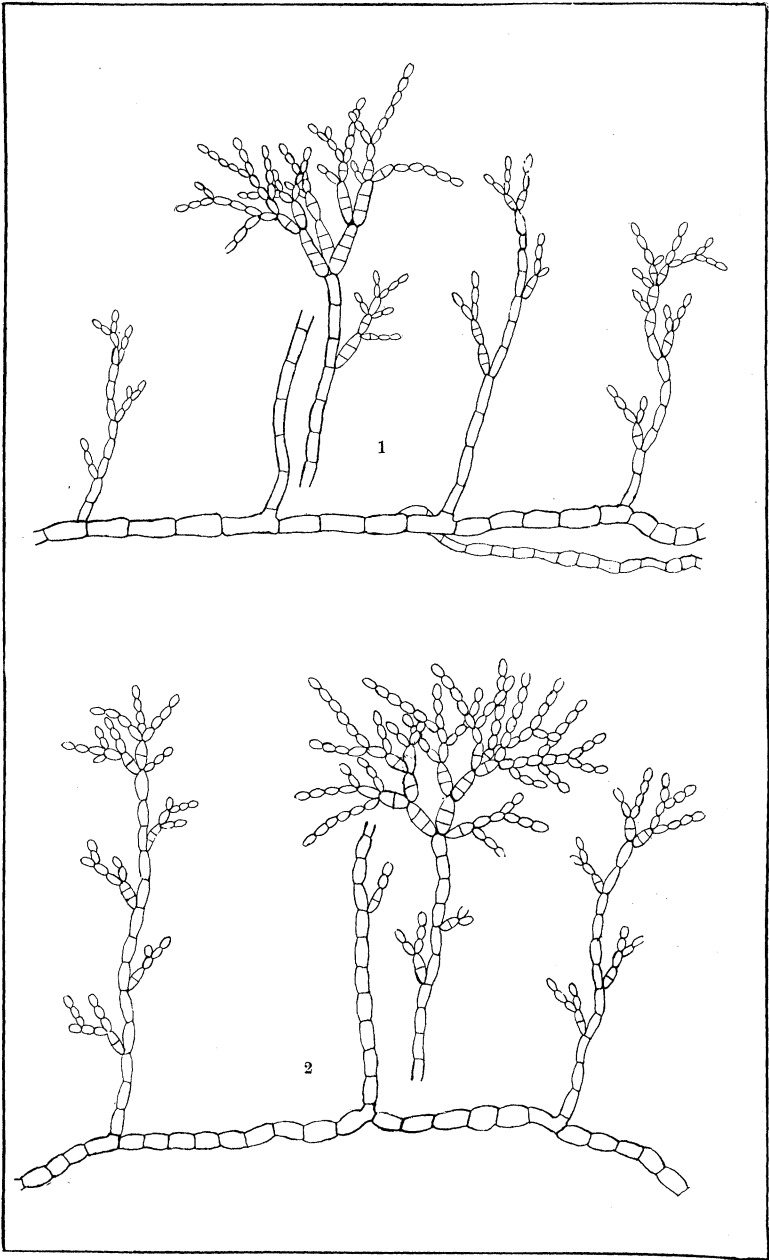


GRAPHIUM.

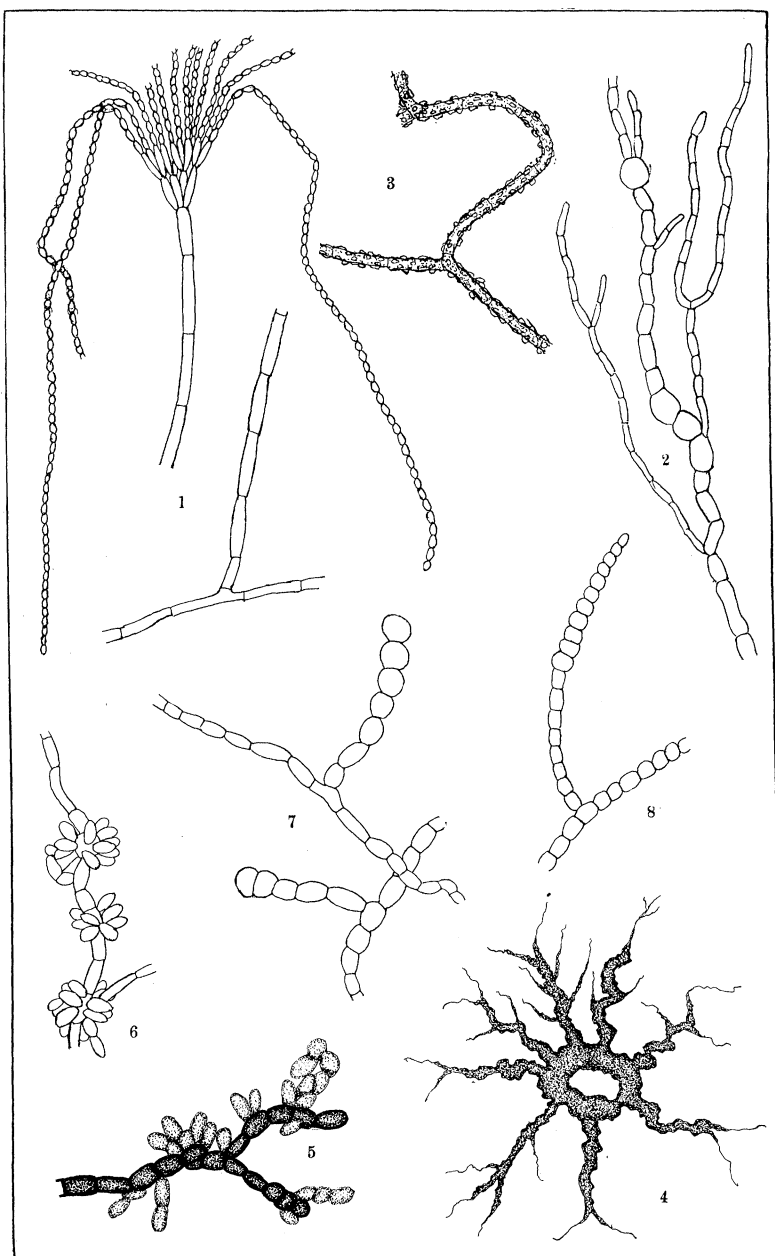


GRAPHIUM.

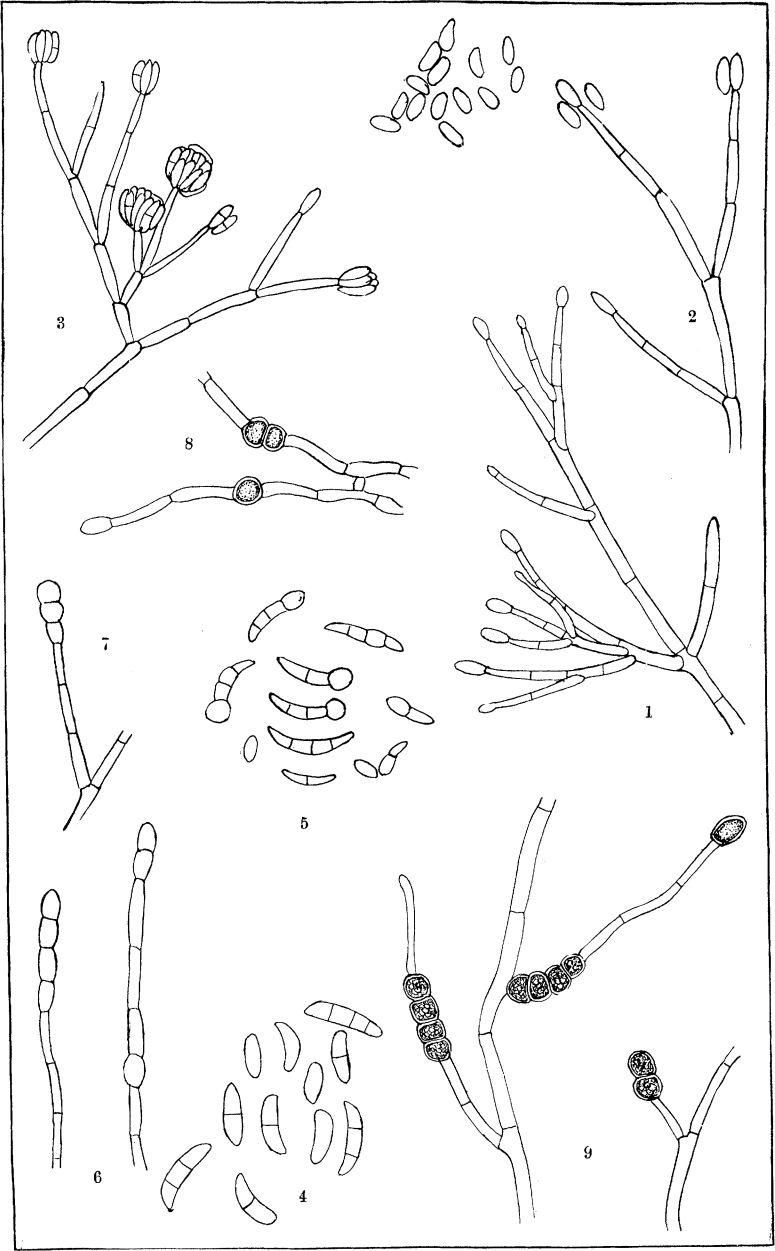




HORMODENDRON.



PENICILLIUM AND HORMISCIMUM.



FUSARIUM ROSEUM.